

# Toxoplasmosis as a public health issue in Serbia

## *Toxoplasmose como assunto de saúde pública na Sérvia*

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### ABSTRACT

**Aims:** To review the significance of toxoplasmosis as a public health issue in Serbia, and analyze the current strategies to alleviate the burden of the disease. **Source of data:** Relevant clinical and epidemiological reports from Serbia published since 1975. **Summary of findings:** Despite 50 years of continuous work on *Toxoplasma gondii* and toxoplasmosis in Serbia, exact data on the frequency of acute clinical disease, acute infections in pregnancy and congenital infection in the offspring are still lacking, due to the vague legal provision that toxoplasmosis is subject to reporting “in case of epidemiological indications”. What, however, is clear is that the major *Toxoplasma*-induced public health issue in Serbia, like elsewhere in Europe, is congenital toxoplasmosis. Continuous monitoring of particular patient groups showed a dramatic decrease in the prevalence of infection over the past two decades, and a consequently increased proportion of women susceptible to infection in pregnancy, suggesting a potential increase in the incidence of congenital toxoplasmosis. Studies of risk factors for infection transmission have provided data to guide national health education campaigns. **Conclusions:** It is expected that the recent appointment of the National Reference Laboratory for Toxoplasmosis as the focal point for the collection of data from the primary level, will provide the means for accurate assessment of the measure of the problem, which is a prerequisite of an evidence-based nation-wide prevention program. In the meantime, health education of all pregnant women, focused at risk factors of major local significance, is advocated as a sound and financially sustainable option to reduce congenital toxoplasmosis.

**Keywords:** *Toxoplasma gondii*; TOXOPLASMOSIS/epidemiology; TOXOPLASMOSIS/prevention & control; TOXOPLASMOSIS, CONGENITAL/prevention & control; TOXOPLASMOSIS, CEREBRAL; PREGNANCY COMPLICATIONS; IMMUNOCOMPROMISED HOST; OPPORTUNISTIC INFECTIONS / HIV related; PUBLIC HEALTH; RISK FACTORS; HEALTH EDUCATION; REFERENCE CENTERS; SERBIA.

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### INTRODUCTION

*Toxoplasma gondii* is one of the most successful parasites on Earth. Known for a century, both the organism and the infection it causes, toxoplasmosis, have been studied in Serbia half this time, ever since the introduction of the Sabin-Feldman test at the Institute for Medical Research (IMR) in 1959. This has long been the single laboratory in ex-Yugoslavia to perform the diagnosis of toxoplasmosis, until commercial tests

made it possible for a number of clinical and public health services, and more recently, laboratories in the private sector, to start performing toxoplasmosis serology. However, these provide only screening while the single facility to perform specialized diagnostic procedures such as prenatal diagnosis including bioassay and molecular diagnosis of parasite DNA, or diagnosis in immunosuppressed individuals, remains the IMR one, for which reasons it has recently been appointed the National Reference Laboratory for Toxoplasmosis (NRLToxo). Despite continuous efforts, clinical research has been largely limited by the legal provision that toxoplasmosis is subject to reporting “in case of epidemiological indications”, the vagueness of which resulted in the anecdotal nature of the data gathered, and a consequential lack of exact

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data on the frequency of acute clinical disease, acute infections in pregnancy and congenital infection in the offspring. These, in turn, are needed as a basis for a nation-wide evidence-based public health program for the prevention of toxoplasmosis.

In this review, current data on the epidemiology of toxoplasmosis in Serbia are presented, along with current public health strategies to decrease the detrimental consequences.

## **TOXOPLASMOSIS AS A CAUSE OF PERINATAL INFECTION**

Toxoplasmosis has long been known as a major cause of perinatal morbidity. Acute infection in pregnancy may lead to fetal infection and subsequent fetal loss or birth of a manifestly or latently infected infant. In a study of the role of toxoplasmosis in the pathological outcome of pregnancy in Serbia,<sup>1</sup> 2,108 women with unsuccessful pregnancies, including 1,747 with spontaneous abortion, 187 with preterm delivery, 126 who delivered stillborn infants, and 48 whose offspring presented with clinical signs at birth, were tested for *Toxoplasma* IgG and IgM antibodies during pregnancy and/or up to 6 weeks after its termination. A nonpregnant group consisting of 157 women and a group of 568 women with a normal pregnancy were included as controls. Acute infection during pregnancy was associated with preterm delivery, stillbirth, and birth of infants with abnormal cranial volume, whereas there was no such association between acute toxoplasmosis and spontaneous abortion, or chronic infection and any of the pathological entities.

As realized back in 1973, the problem with toxoplasmosis is to determine how much of a problem it really is,<sup>2</sup> implying wide variations from country to country, depending on geographical region, socioeconomic and cultural features. In an attempt to determine the extent of the problem of congenital toxoplasmosis in our country, we performed a retrospective analysis of acute toxoplasmosis in pregnancy and of congenital toxoplasmosis in the neonate/infant over the period 1994-1998.<sup>3</sup> The results showed a rather alarming discrepancy between the detection of acute infection in pregnancy and sequential neonatal follow-up on the one hand, and the occurrence of congenital infection in infants born to mothers unsuspected of infection (and therefore not tested) on the other. This was further emphasized by the fact that only 2.5% women in this series were tested prior to pregnancy as a preventive measure. Thus, this data suggested urgent implementation of prevention programs against this potentially detrimental perinatal

infection, thought to lead to adverse sequelae in up to 100% of those affected.<sup>4</sup>

## **EPIDEMIOLOGY OF TOXOPLASMOSIS IN PREGNANT WOMEN IN SERBIA**

Long-term continuous epidemiological surveillance of toxoplasmosis in Serbia shows an intensive dynamics. Two decades of a prevalence of around 50%,<sup>5</sup> and a subsequent increase to an all-time high of up to 80% in the eighties,<sup>1,6</sup> were followed by a gradual decrease to a present prevalence of 30-35%.<sup>7</sup> Such infection dynamics is not a peculiarity of Serbia; in all of Europe a decrease has been observed over the past decades, such as in France from 84% in 1965 to 67% in 1988<sup>8</sup> to 54% in 1995,<sup>9</sup> or closer to Serbia, in northern Greece, from 37% in 1984 to 24% in 2004.<sup>10</sup>

In Europe, significant variations have been shown to occur not only between countries but also within a given country, indicating local variations in the influence of epidemiological factors contributing to infection. Thus, European countries have implemented prevention programs in measure with the respective estimated risk of congenital toxoplasmosis. In France, where this risk was long known to be high, a national screening-in-pregnancy program has been established since 1978, while for instance in Great Britain, based on a low infection incidence, only nation-wide health education of pregnant women was recommended.<sup>11</sup> In Serbia, a general screening-in-pregnancy program could never be afforded due to its cost *versus* the financial capabilities of the public healthcare system. As an alternative to general screening, we considered that insight into the risk factors of particular local significance could improve the quality of and the compliance with the hygienic and dietetic advice given to pregnant women as a preventive measure, as well as identify particular subpopulations at an increased risk of infection, who could then be selectively screened. To this effect, two studies of the risk factors for *Toxoplasma* infection in generative age women have been performed, which despite some differences generated similar results.<sup>6,12</sup> The first one,<sup>6</sup> carried out between 1988 and 1991 in a series of 1,157 women from the Belgrade area, showed an overall prevalence of *Toxoplasma* infection of 77%, but which was decreasing significantly over the study period ( $p < 0.01$ ). Factors significantly associated with infection included age [relative risk (RR)=1.18, 95% confidence interval (CI)=1.02-1.37,  $p=0.022$ ], undercooked meat consumption (RR=2.22, 95%CI=1.2-2.86,  $p=0.001$ ), and the year of entry into the study (RR=0.69, 95%CI=0.6-0.8,  $p=0.000$ ). In addition, exposure to soil (farming, gardening) was

significantly associated with infection only in women below age 20 (RR=1.38, 95%CI=1.12-1.97, p=0.037). Since cats are the single source of *Toxoplasma* oocysts, this finding indicated that cats were an epidemiologically significant source of environmental contamination in Belgrade. However, cat ownership itself was not associated with infection. A later study of risk factors for *Toxoplasma* infection in a series of 2,936 women aged 15-49 years from throughout Serbia performed between 1988 and 1997 showed an overall prevalence of infection of 69%, but with very wide variations both over time (decreasing from 86% in 1988 to 39% in 1997) and region (40-90%).<sup>12</sup> The risk of infection increased from the south to the north (RR=0.97, CI=0.94-0.98). Within Belgrade, the risk was higher in urban than in suburban zones (RR=0.79, CI=0.64-0.93). The single infection transmission factor shown to be a predictor of infection in the whole study group was undercooked meat consumption (RR=1.6, CI=1.2-2.1), while exposure to soil was a predictor only in women aged 15-19 (RR=10.3, CI=2.7-38.6). Owning pet cats had no influence. Furthermore, while *Toxoplasma* infection was not associated with pathological pregnancies (p=0.51) in the whole study population, it was significantly associated with pathological pregnancies in women exposed to both undercooked meat consumption (p=0.009) and in those aged 15-19 in contact with soil (p=0.022), as well as in women residing in highly urban communities (p=0.048). These findings led us to conclude that the dramatic decrease in the prevalence of *Toxoplasma* infection over the nineties resulted in a rising proportion of women exposed to infection in pregnancy and consequently in an increased risk of congenital toxoplasmosis. Based on these data, and bearing in mind that the public health system could not afford a general screening-in-pregnancy program, we proposed health education of all pregnant women in combination with serological testing of those exposed to predictors of infection as an epidemiologically sound and financially sustainable alternative.<sup>12</sup>

Finally, in a series of 765 women of generative age from throughout Serbia tested between 2001 and 2005, in which the prevalence of infection was 33%, the infection risk factors were analyzed in a case-control manner in a group of 53 women with acute infection (cases) compared to a group of seronegative women matched for age and education level (controls).<sup>7</sup> Undercooked meat consumption was the single predictor of infection in women with acute infection, with an 11-fold increased risk of infection in women who acknowledged consumption of undercooked meat (RR=11.21, 95% CI=3.10-40.53, P=0.000).

Since these studies repeatedly showed undercooked meat consumption to be the most significant predictor of *Toxoplasma* infection in Serbia, we analyzed the significance of the consumption of particular meat types as sources of infection.<sup>7</sup> Of the meat types mostly consumed in Serbia, only consumption of beef (RR=1.006, 95% CI=1.001-1.011, P=0.027) was shown to influence *Toxoplasma* infection rates.

The above data led us to look into the frequency of toxoplasmosis in most commonly used meat animals in our milieu. A cross-sectional survey on the seroprevalence of *Toxoplasma* infection in a representative sample of cattle, sheep and pigs from different regions of Serbia conducted between June 2002 – June 2003 showed a seroprevalence of 76.3% in cattle, 84.5% in sheep and 28.9% in pigs, showing that they present a significant reservoir of *Toxoplasma* infection for humans.<sup>13</sup>

Another confirmation for the risk of undercooked meat consumption for *Toxoplasma* infection is provided by a recent analysis of the seasonal distribution of acute toxoplasmosis in Serbia.<sup>14</sup> This showed that in both symptomatic (recent lymphadenopathy) and asymptomatic patients (women tested for obstetric reasons) acute infection showed significant (p<0.0001) seasonality, related to the seasons of the year (p<0.0001). In the symptomatic patients, acute infections occurred more often between October and March (p=0.0486). Although more asymptomatic acute infections were diagnosed between February and July (p=0.0037), low IgG avidity suggests infection had occurred within the previous trimester (between November and April). Undercooked meat consumption was shown as a risk factor for symptomatic infection in the October-March period (OR 7.67, 95% CI 1.61-36.45) which may be associated the cultural habits of eating more meat in the winter period, particularly on the occasions of family festivities frequent at this time on the year, when young whole pigs and lambs are roasted.

## **TOXOPLASMOSIS AS AN OPPORTUNISTIC INFECTION**

The significance of *Toxoplasma* as an opportunistic agent has been recognized with the increase in the number of immunosuppressed individuals, such as patients with malignant and systemic diseases treated with immunosuppressive drugs, or in organ transplant recipients, but particularly with the outbreak of AIDS, as reviewed elsewhere.<sup>15</sup>

Once infected, the host acquires lifelong immunity induced by the persistence of the parasite in an encysted form. However, in the absence of immunological

control, reactivation of a previously latent infection may occur, resulting in a wide clinical spectrum, predominantly within the central nervous system. Disease due to *Toxoplasma* is the leading cause of focal cerebral lesions in patients with AIDS.<sup>16</sup> The prevalence of latent toxoplasmosis in any given region indicates the local exposure to infection reactivation. One study to determine the frequency of and risk factors for toxoplasmic encephalitis (TE) in AIDS patients in Serbia (within the then-Yugoslavia), performed between 1991 and 1995, showed *Toxoplasma* IgG antibodies in 127 of the 288 AIDS patients (44.1%) tested serologically (including all with neurological abnormalities).<sup>17</sup> A total of 31 patients developed TE, indicating an overall TE attack rate of 7.8%. Of these, 29 TE episodes occurred in the *Toxoplasma*-seropositive patients, but two were registered in the seronegative ones. The cumulative incidence of TE in the seropositive patients was 32.7% for 60 months (30% for 24 months). While not associated with age, sex or HIV transmission risk factor, the risk for TE increased with the decrease in the CD4+ T cell count [relative risk (RR)=0.276, 95% confidence interval (CI)=0.198-0.673, p=0.0013], and was reduced in patients on systemic PCP prophylaxis (RR=0.222, 95%CI=0.068-0.642, p=0.006). These results indicated that in areas of high exposure to *Toxoplasma* such as Serbia, serology for toxoplasmosis in HIV-positive patients should be performed early after the diagnosis of HIV infection, before it has developed into AIDS. However, the situation has largely changed since the introduction of HAART in 1996, after which opportunistic infections including toxoplasmosis are seen extremely rarely.<sup>18</sup>

## **PUBLIC HEALTH STRATEGIES TO ALLEVIATE THE BURDEN OF THE PROBLEM**

As part of the process of reform of public health services in Serbia, a network of reference laboratories for infectious diseases of public health interest was established by the Ministry of Health of Serbia in 2008. The appointed National Reference Laboratory for toxoplasmosis (NRLToxo) should become the focal point for the collection of data from primary level laboratories, which will allow for accurate assessment of the size of the problem of toxoplasmosis in Serbia. Such data are necessary if prevention programs are to be planned. The remarkable decrease in *Toxoplasma* seroprevalence over the past two decades to the present ~30% resulted in an increased proportion of women susceptible to infection in pregnancy. Moreover, in line

with the global climate changes, and with a change of the climate in Serbia *versus* one more similar to that in the more southern countries of Macedonia and Greece, and given the data on a north-to-south decrease in infection rates in the region (from Hungary via Serbia to Greece),<sup>19,10</sup> a further decrease in the prevalence of *Toxoplasma* infection may be expected in Serbia. And actually, the NRLToxo currently witnesses an increasing number of acute cases as well as of congenital transmissions, reflecting the shortcomings of the healthcare system to identify all infections in pregnancy and intervene appropriately. What is now needed is an organizational algorithm indicating the responsibilities of the primary level laboratories and the NRLToxo, as well as implementation of a quality assurance program. One step in the desired direction is the publication of the Good Practice Guide currently in preparation.

## **CONCLUSIONS**

Congenital toxoplasmosis is the major *Toxoplasma*-induced public health issue in Serbia. In view of the decrease in the prevalence of *Toxoplasma* infection which has occurred in Serbia over the two past decades, and the consequently increased proportion of women exposed to infection during pregnancy, implementation of a nation-wide program for the prevention of congenital toxoplasmosis is warranted. A prerequisite for such a program to be cost-effective is accurate assessment of the annual number of congenital toxoplasmosis cases, expected to be provided by the recently appointed NRLToxo. In the meantime, health education of all pregnant women focusing particularly on the locally most significant risk factors for infection transmission, is a sound and financially sustainable option for the prevention of congenital toxoplasmosis in Serbia.

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## **REFERENCES**

1. Djurković-Djaković O. *Toxoplasma* infection and pathological pregnancy. *Gynecol Obstet Invest.* 1995;40:36-41.
2. Fleck DG. The problem of congenital toxoplasmosis. In: *Intrauterine infections.* Amsterdam: Elsevier; 1973. p.45-52. [Ciba Found Symp. (new series). 10]
3. Djurković-Djaković O, Nikolić T, Ljubić A, et al. Toxoplasmosis as a perinatal infection in Yugoslavia at the end of the millennium. *Acta Infectol Jugosl.* 1998;3:123-8.
4. Wilson CB, Remington JS, Stagno S, et al. Development of adverse sequelae in children born with subclinical congenital *Toxoplasma* infection. *Pediatrics.* 1980;66:767-74.

5. Šibalić D, Radović M, Šulović V. Infection par *Toxoplasma gondii* pendant la grossesse et avant elle et les conséquences possibles sur le fœtus. In: Petrović Z, editor. Proceedings of the Second European Multicolloquy of Parasitology; 1975 Sep 1-6; Trogir, Yugoslavia; 1975. p.23-8.
6. Bobić B, Jevremović I, Marinković J, et al. Risk factors for *Toxoplasma* infection in a reproductive age female population in the area of Belgrade (Yugoslavia). *Eur J Epidemiol.* 1998;14:605-10.
7. Bobić B, Nikolić A, Klun I, et al. Undercooked meat consumption remains the major risk factor for *Toxoplasma* infection in Serbia. *Parassitologia.* 2007;49:227-30.
8. Jeannel D, Niel G, Costagliola D, et al. Epidemiology of toxoplasmosis among pregnant women in the Paris area. *Int J Epidemiol.* 1988;17:595-602.
9. Ancelle T, Goulet V, Tirard-Fleury V, et al. La toxoplasmose chez la femme enceinte en France en 1995. *BEH Bull Epidemiol Hebdom.* 1996;(51):227-9. [citado 2010 Jan 5]. Disponível em: [http://www.invs.sante.fr/beh/1996/9651/beh\\_51\\_1996.pdf](http://www.invs.sante.fr/beh/1996/9651/beh_51_1996.pdf)
10. Diza E, Frantzidou F, Souliou E, et al. Seroprevalence of *Toxoplasma gondii* in northern Greece during the last 20 years. *Clin Microbiol Infect.* 2005; 11:719-23.
11. Henderson JB, Beattie PC, Hale EG, et al. The evaluation of new services: possibilities for preventing congenital toxoplasmosis. *Int J Epidemiol.* 1984;13:65-72.
12. Bobić B, Nikolić A, Djurković-Djaković O. Identification of risk factors for *Toxoplasma gondii* infection in Serbia as a basis of a program for prevention of congenital toxoplasmosis. *Srp Arh Celok Lek.* 2003;131:162-7.
13. Klun I, Djurković-Djaković O, Katić-Radivojević S, et al. Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: seroprevalence and risk factors. *Vet Parasitol.* 2006;135:121-31.
14. Bobić B, Klun I, Nikolić A, et al. Seasonal variations in *Toxoplasma* seroprevalence in Serbia. *Vector Borne Zoonotic Dis.* In press 2010.
15. Djurković-Djaković O. Toxoplasmosis and immunosuppression. *Srp Arh Celok Lek.* 1998;126:197-203.
16. Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. *Clin Infect Dis.* 1992;15:211-2.
17. Djurković-Djaković O, Bobić B, Vuković D, et al. Risk for toxoplasmic encephalitis in AIDS patients in Yugoslavia. *Int J Infect Dis.* 1997;2:74-8.
18. Jevtović DJ, Salemović D, Ranin J, et al. The prevalence and risk of immune restoration disease in HIV-infected patients treated with highly active antiretroviral therapy. *HIV Med.* 2005;6:140-3.
19. Szenasi Z, Ozsvar Z, Nagy E, et al. Prevention of congenital toxoplasmosis in Szeged, Hungary. *Int J Epidemiol.* 1997; 26:428-35.