

RISK FACTORS FOR *TOXOPLASMA* INFECTION IN PREGNANT WOMEN IN FYR OF MACEDONIA¹

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Summary:

The aim of the study was to identify risk factors for *Toxoplasma gondii* infection in pregnant women in FYR of Macedonia. Retrospective analysis of serological and epidemiological data in a series of 235 pregnant women from Macedonia, tested for *Toxoplasma* infection between January 2004 and December 2005, showed an overall prevalence of infection of 20.4%. Exposure to transmission factors significantly increased the risk of infection (RR = 1.989, 95% CI = 1.041-3.800, $p = 0.037$). The single infection transmission factor that was a predictor of infection in the whole series was exposure to soil (RR = 1.946, 95% CI = 1.026-3.692, $p = 0.041$). Based on prevalence and the established risk factors for *Toxoplasma* infection in Macedonia, the health education programme as a sustainable measure for the prevention of congenital toxoplasmosis should focus on educating women of generative age to avoid contact with soil (farming, gardening), and/to adhere to strict hygienic practices afterwards.

KEY WORDS: *Toxoplasma gondii*, infection, pregnant women, risk factors, seroepidemiology, Macedonia.

Résumé : FACTEURS DE RISQUE D'INFECTION PAR LE *TOXOPLASME* CHEZ LA FEMME ENCEINTE EN MACÉDOINE

Le but de l'étude était d'identifier des facteurs de risque d'infection par *Toxoplasma gondii* chez la femme enceinte en Macédoine. Une analyse rétrospective des données sérologiques et épidémiologiques portant sur l'infection par le toxoplasme a été menée chez de 235 femmes enceintes macédoniennes suivies entre janvier 2004 et décembre 2005. La prévalence de l'infection était de 20,4%. L'exposition aux facteurs de risque augmente significativement les taux d'infection (RR = 1,989, IC 95% = 1,041-3,800, $p = 0,037$). Le seul facteur de risque prédictif de la transmission de l'infection, retrouvé dans l'ensemble de l'échantillon, a été l'exposition au sol (RR = 1,946, IC 95% = 1,026-3,692, $p = 0,041$). À la lumière de ces données épidémiologiques, le programme de prévention à long terme de la toxoplasmose congénitale devrait se concentrer sur les femmes éduquées, en âge de procréer, afin qu'elles évitent le contact avec le sol (agriculture, jardinage), et qu'elles adhèrent aux mesures d'hygiène qui en découlent.

MOTS CLÉS : *Toxoplasma gondii*, infection, femme enceinte, facteurs de risque, séroépidémiologie, Macédoine.

Toxoplasmosis is a globally distributed zoonosis caused by the ubiquitous protozoan *Toxoplasma gondii*. In addition to being an opportunistic infection capable of inducing serious pathology in immunosuppressed individuals, the clinical significance of toxoplasmosis remains defined by its role in perinatal pathology; maternal primary infection during pregnancy may result in foetal infection, causing potentially serious congenital toxoplasmosis.

Prevention of congenital toxoplasmosis implies prevention of maternal periconceptual infection.

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Approaches to prevention basically vary between a general screening-in-pregnancy programme and education on how to avoid *Toxoplasma* infection in pregnancy. The choice of cost-effective strategy is based on the estimated risk of congenital toxoplasmosis in a particular population, which largely depends on the infection prevalence and incidence rates. Common practices are such that countries with a low prevalence of infection generally opt for health education (Henderson *et al.*, 1984; Ho-Yen *et al.*, 1995; Gilbert & Pecham, 2002) while those with a high prevalence adopt screening-in-pregnancy programmes (Kravetz & Federman, 2005; Thulliez, 1992)

In FYR of Macedonia, no systematic programme for the prevention of congenital toxoplasmosis has ever been implemented. Since a screening-in-pregnancy programme is presently not financially sustainable, the alternative option is health education of all pregnant women. To adapt the health education programme to the local epidemiological situation, we conducted a laboratory-based study to identify risk factors for *Toxoplasma* infection in pregnant women from throughout FYR of Macedonia examined in 2004 and 2005.

MATERIAL AND METHOD

SUBJECTS AND DATA COLLECTION

A cross-sectional study of pregnant women (age 15-45 years) who presented for serology at the Institute of Microbiology and Parasitology, School of Medicine of Skopje during the 2-year period between 1 January 2004 and 31 December 2005 was conducted. The women were referred to the laboratory for a routine pregnancy test by their gynaecologists, mostly from the region of Skopje. Upon presentation, all women were interviewed using a standardized questionnaire, which included questions on age (stratified into six five-year groups), degree of education (modalities: grade school, secondary or university level), and history of pathological pregnancy (spontaneous abortion, stillbirth, pre-term delivery), as well as on life-style habits pertaining to infection transmission risk factors: consumption of undercooked meat, exposure to soil (townhouse with garden, farming and gardening in suburban home), and exposure to cats (pet cat ownership). Inclusion criteria consisted of current pregnancy, response to all questionnaire items and *Toxoplasma* serology. The final series involved 235 women who all gave consent for participation in the study, and the study was approved by the University of Skopje School of Medicine.

SEROLOGY

Specific anti-*Toxoplasma* IgG antibodies were detected by the ELISA technique (IMMULITE, Diagnostic Products Corporation, Los Angeles, CA, USA). The cut-off was 10 IU/mL.

STATISTICS

All data were analyzed using a SPSS (version 10.0) statistical package. The differences in the rates of *Toxoplasma* infection between the two years of study and between the youngest and oldest age group were analyzed by the Chi-square test or Fisher's exact test (as applicable). The association between *Toxoplasma* infection and the demographic and epidemiological parameters taken as variables was analyzed by the Wald forward stepwise logistic regression method, using both univariate and multivariate approaches. The dependent variable was the presence of infection (infected / non-infected), and all other variables were considered independent. The adequacy of the logistic model was estimated at a level of 10 %, and the level of statistical significance was < 5 %.

No. of <i>Toxoplasma</i> seropositive (%)	Univariate analysis		Multivariate analysis	
	RR (95 % CI)	p	RR (95 % CI)	p
Demographic factors (n = 235)				
age group		1.129 (0.885-1.679)		0.226
15-19 (n = 11)	2 (18.18)			
20-24 (n = 52)	7 (13.46)			
25-29 (n = 111)	24 (21.62)			
30-34 (n = 43)	11 (25.58)			
35-39 (n = 15)	3 (20.00)			
> 40 (n = 3)	1 (33.33)			
degree of education		0.597 (0.359-0.992)		0.046
grade school (n = 26)	10 (38.46)			
secondary level (n = 130)	25 (19.23)			
university level (n = 79)	13 (16.46)			
Transmission risk factors (n = 235)				
consumption of undercooked meat		2.427 (0.559- 10.534)		0.237
yes (n = 7)	2 (28.57)			
no (n = 228)	45 (19.73)			
exposure to soil		1.946 (1.026- 3.692)		0.042
yes (n = 92)	25 (27.17)		1.946 (1.026- 3.692)	0.042
no (n = 143)	23 (16.08)			
exposure to cats		0.719 (0.281- 1.837)		0.491
yes (n = 37)	6 (16.22)			
no (n = 198)	42 (21.21)			

Table I. – Association of *Toxoplasma* infection rates and epidemiological variables (logistic regression).

RESULTS

The overall seroprevalence of *Toxoplasma* infection in the series of 235 pregnant women aged 15 to 45 years tested in 2004 and 2005 was 20.4%. The difference in the seroprevalence of *Toxoplasma* infection between the two years of study (19.2% – 14/73 in 2004; 21% – 34/162 in 2005) was not significant ($\chi^2 = 0.101$, $p = 0.750$).

The seroprevalence of *Toxoplasma* infection according to demographic and epidemiological data is presented in Table I. Exposure to all transmission factors taken together significantly increased the risk of infection (RR = 1.989, 95% CI = 1.041-3.800, $p = 0.037$). Univariate analysis of individual demographic and epidemiological variables as risk factors for *Toxoplasma* infection showed that infection was significantly associated with the degree of education and exposure to soil. Despite an age-associated increase in the prevalence of infection, this increase was not significant, not even between the youngest and oldest age groups ($p = 0.546$).

Factors shown to be associated with infection by univariate analysis (degree of education and exposure to soil) were included as independent variables in the multivariate regression model. The analysis determined that exposure to soil was the only independent risk factor i.e. predictor of infection in the studied population (Table D). The risk of infection was significantly (RR = 1.946, 95% CI = 1.026-3.692, $p = 0.042$) increased in women who were exposed to soil. Further analysis determined that exposure to soil was not associated with the degree of education ($p = 0.063$), confirming that exposure to soil was not a confounding factor. Further analysis of the infection in the group of women who were not exposed to soil, showed that the risk of infection was not associated ($p > 0.05$) with any of the studied demographic and epidemiological variables.

Toxoplasma infection was not associated with a history of previous pregnancy ($p = 0.727$). In women with previous pregnancy/ies, infection was not associated with a history of pathological pregnancy ($p = 0.940$) (preterm delivery, $p = 0.816$; stillbirth, $p = 0.872$; spontaneous abortion, $p = 0.872$). Even in the women with exposure to soil and a history of previous pregnancies, *Toxoplasma* infection was not associated with a history of pathological pregnancy ($p = 0.872$) or any of its entities ($p > 0.05$).

DISCUSSION

This study of *Toxoplasma* infection in a population of 235 pregnant women in FYR of Macedonia between 2004 and 2005 determined a seroprevalence of 20.4%. In a previous study from the same

laboratory carried out between 1998-2000 in a series of 400 women of reproductive age (200 pregnant and 200 non-pregnant), the seroprevalence was 25.5% (Cvetkovic *et al.*, 2003). While the representativeness of both studied series may be questioned vs. the number of pregnant women in the country, they are comparable in that the patients were referred from the same territory (by the same gynecologists), and with no selection criteria (other than completion of the questionnaire). Thus, the results of this study suggest a decrease in the prevalence of *Toxoplasma* infection in FYR of Macedonia in this decade. Such a decrease in FYR of Macedonia may be regarded as part of a changing pattern of *Toxoplasma* infection in the Western Balkan region, with one dimension of this pattern being spatial (a decrease of prevalence from north to south), and the other temporal (a decrease of prevalence in the last 20 years). This pattern is evident when comparing studies from countries neighbouring Macedonia both to the north and to the south (Serbia: 86% in 1988, 53% in 1994, 33% in 2004; Greece: 36% in 1984, 26% in 1994, 20% in 2004) (Bobić *et al.*, 2003, 2007; Diza *et al.*, 2005). Furthermore, a decreasing trend of *Toxoplasma* infection noted in the Western Balkan region is probably a part of a wider changing pattern of *Toxoplasma* infection in Europe, as it has been reported in many countries, including Sweden, France, Austria and Slovenia (Forsgren *et al.*, 1991, Jeannel *et al.*, 1988; Berger *et al.*, 2009; Aspöck & Pollak, 1992; Logar *et al.*, 1995; Logar *et al.*, 2002.). Of the socio-demographic and transmission risk factors examined as potential risk factors for *Toxoplasma* infection, exposure to soil has been determined as the single predictor of *Toxoplasma* infection. The significance of exposure to soil as a risk factor for *Toxoplasma* infection is well-known (Hall *et al.*, 2001; Hough, 2007), and it raises the issue of the significance of cats as an infection source; since, however, this study did not show pet cat ownership to be a risk factor, environmental contamination with oocysts by the stray cat population should be considered. It must be noted, however, that this analysis of risk factors may be limited by the type of study itself, which although generally used, is less accurate than a case-control study, and also, by the broadness of the questionnaire. Namely, although undercooked meat *per se* was not shown to be a risk factor, the refinement of the questionnaire to include detailed modalities of exposure to undercooked meat such as eating out frequently, eating fast food based on minced meats, washing of kitchen utensils when preparing food at home, might eventually expose this factor as significant. Concerning the role of cats, however, another frequently neglected question is consumption of insufficiently washed fruits and vegetables.

Toxoplasma infection was not a significant cause of pathological pregnancies in FYR of Macedonia. As much as the decrease in *Toxoplasma* infection rates may at

first glance seem desirable, it may be anticipated that the consequential increase in the pool of seronegative women entering childbearing age would lead to an increase in the incidence of congenital toxoplasmosis (Olesen-Larsen & Lebech, 1994). Indeed, in Austria, the decrease in *Toxoplasma* prevalence from 50 to 37 % during the eighties has resulted in an increase from 0.4 to 0.8 % in the incidence of primary infections in pregnancy (Aspöck & Pollak, 1992). Similarly, in Slovenia, the decrease in *Toxoplasma* seroprevalence from 52 % recorded in the early eighties to 34 % in the nineties has been associated with an increased incidence of acute infections in pregnancy, from 0.33 % in the nineties to 0.94 % at the turn of the century (Logar *et al.*, 1995, 2002). Increased risk of congenital toxoplasmosis at times of changing patterns of *Toxoplasma* infection warrants implementation of a systematic programme of prevention of congenital toxoplasmosis.

A financially sustainable prevention programme is health education of all pregnant women and those entering reproductive age. A prerequisite for such a programme is to be well adapted to the local epidemiological situation i.e. to take into account the locally dominant risk factors for *Toxoplasma* infection (Hall *et al.*, 2001). Based on the above results, the health education programme in FYR of Macedonia should focus on educating all women of generative age on how to avoid or minimize the risk from direct contact with soil by adhering to some simple but strict hygienic measures, including wearing gloves when farming or gardening, and thorough washing of hands after contact with soil.

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