Toxoplasmosis in Kosovo pregnant women

Pietro Dentico[1], Anna Volpe[1], Giovanni Putoto[2], Naser Ramadani[3], Luigi Bertinato[2], Merita Berisha[3], Nicola Schinaia[4], Gianluca Quaglio[2], Paolo Maggi[1]

[1]*Department of Internal Medicine, Immunology and Infectious Diseases, University of Bari, Bari, Italy;*
[2]*Veneto Region, Italian Co-operation, Peja-Pec Hospital Training Project Team, Venice, Italy;*
[3]*National Institute of Public Health, Kosova;*
[4]*Epidemiology Unit, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*

SUMMARY

This study presents the initial results of a collaborative project aimed at the evaluation of *Toxoplasma* seroprevalence in a population of Kosovar pregnant women. The serum samples of 334 pregnant women were tested to detect IgG, IgM, IgG avidity for toxoplasmosis. Data regarding age, occupation, area of origin and education were also obtained for the pregnant women examined; 97/334 (29.4%) resulted positive for IgG antibodies, four of whom (4.1%) were also positive for IgM, (1.2% of the total population examined). All four IgM-positive pregnant women also demonstrated low avidity tests.

The rate of IgG seroprevalence found in our study was lower than that observed in various European countries, especially those of western Europe. Conversely, the percentage of recent infections was higher than expected. The higher rate of infections could be the result of a recent toxoplasmosis epidemic in Kosovo, most likely due to the altered hygienic conditions caused by the forced transfer of the ethnic-Albanian population from an area of low (Serbia) to high (Kosovo) toxoplasmosis prevalence.

KEY WORDS: Pregnancy, Toxoplasmosis, Serological test, IgG, IgM, Pre-gravidic screening

INTRODUCTION

Kosovo was under the administration of the United Nations Security Council from 1999 to 2008. Kosovo declared independence in 2008 and recently the area attained national autonomy, with some limitations of sovereignty and with the support of the European Union Rule of Law Mission and a NATO military force to insure a continuation of international civil presence in the area (Bilefsby, 2008).

The population, estimated at 1.9 million, is one of the youngest populations in Europe and has suffered substantially over the past decade from economic, social and political turmoil related to the fragmentation of Yugoslavia in the early 1990s and the ensuing armed conflict in 1999. Approximately 37% of the population live in poverty and unemployment is estimated at 40%. About 60% of the population live in rural areas (World Bank, 2005) which often lack adequate hygienic and sanitary conditions. The health care system was greatly damaged and neglected as a result of the conflict, especially services regarding maternal health during pregnancy and newborn children. Currently, the public health system is supervised by the University Hospital in Pristina (the capital of Kosovo) and five regional hospitals. However, as its health care system requires further development, the current data on the epidemiology of infectious diseases (i.e. HBV, HCV, HIV) are still incomplete. In particular, toxoplasmosis is a serious problem in various Balkan countries but there is a lack of information regarding the seroprevalence of the infection during pregnancy. At present, a seroprevalence rate greater than 50% has been reported for women in childbearing age throughout Western Europe, Africa and South and Central America with the

Corresponding author
Pietro Dentico
Clinica Malattie Infettive
Piazza Giulio Cesare 11
70124 Bari, Italy
E-mail: p.dentico@clininf.uniba.it

highest infection rates during pregnancy reported in Belgium, Netherlands, Austria, Germany, Czechoslovakia, and some areas of Canada (Williams *et al.*, 1981; Gilbert, 1999; Lebech *et al.*, 1999; Tizard *et al.*, 1977; Bowie *et al.*, 1997). In Belgrade (Serbia), in a retrospective study of risk factors for *Toxoplasma gondii* conducted on a population of 2936 resident females over a nine-year period (1988-1997), the overall infection rate was 69% with wide variations both over time and region (Bobic *et al.*, 1998; Bobic *et al.*, 2003). The frequency of toxoplasmosis acquisition during pregnancy ranges from 1-2/1,000 pregnancies in several countries; moreover, an increased risk of infection has been found in populations moving from an area of low to high prevalence of infection during or before childbearing (Allain *et al.*, 1998; Jenum *et al.*, 1998; Horion *et al.*, 1990). Congenital transmission of toxoplasmosis can occur when a woman acquires the infection during pregnancy and transmits it to her foetus (McCabe *et al.*, 1988). The congenital infection has a prevalence of 0.2-2/1000 births in Europe, and congenital toxoplasmosis affects 1-10,000 newborn children of whom 1% to 24% develop lesions (Wong *et al.*, 1994). In a previous report derived from an epidemiologic survey on pregnant Albanian women, we observed an unexpectedly high percentage of IgM-positivity for toxoplasmosis with respect to the European mean (Maggi *et al.*, 2009). For this reason, we sought to extend our investigation to Kosovo. The study was carried out within the framework of a training project for health workers at Peja Hospital (Peja Hospital Training Project-PHTP) which was performed between 2004 and 2007 with the technical support of the Venetian Regional Health Authority and the Italian Cooperation Agency; a total of 1285 subjects were recruited from different population groups in the Peja region to evaluate the seroprevalence of infectious diseases (HIV, HBV, HCV, syphilis) (Quaglio *et al.*, 2008).

PATIENTS AND METHODS

This cross-sectional study was performed from January 1 to March 30, 2005 and involved all pregnant women admitted for delivery to the Peja Hospital. The study was approved by the Kosovo Institute of Public Health and written informed consent was obtained from each participant. Demographic and clinical data were collected using a structured questionnaire, administered during an interview. The data included age, marital status, education, occupation, work activities abroad, area of residence (urban or rural area), previous surgical procedures, history of hospitalization, transfusion of blood/blood products, and other medical treatment (intramuscular injection, dental care). A blood sample was obtained from each woman and the sera were divided into 3 serum aliquots: 2 aliquots of 2 ml each were sent to Italy for laboratory analyses, and 1 aliquot of 0.5 ml was stored at -20°C in the Peja Hospital Laboratory. Three commercial enzyme immunoassays for toxoplasmosis antibody detection were utilized: *Toxoplasma* IgG EIA Well, *Toxoplasma* IgM EIA Well, and *Toxoplasma* IgG Avidity EIA Well (Radim Diagnostic Pomezia - Italy). All the *Toxoplasma* tests used are based on the enzyme immunoassay method with high sensitivity and specificity. All tests were performed according to the manufacturer's instructions. Statistical analyses were performed on data with the chi-square and Fisher's exact tests to evaluate possible differences between the various diagnostic assays and for other factors, such as occupation, age, and residence. A p-value <0.05 was considered significant.

RESULTS

Patient characteristics including employment, area of origin and degree of education are summarized in Table 1. Of the pregnant women examined, 97/334 (29,4%) were positive for IgG and 4/97 (4.1%) for IgM. All IgM-positive pregnant women also demonstrated low avidity tests. The area of origin and degree of education were not significantly related to the presence of IgG or IgM, while IgM resulted statistically higher among housewives. Subsequently, the women were stratified into three age groups: 17-28 years, 29-38 years and 39-46 years. The distribution and the results are summarized in Table 2. When analyzing these results, it was observed that the prevalence varied according to age group, ranging from 25.30% in the first group (17-28 yrs) to 30.4% in the second group (29-38 yrs) and 50% in the third (39-46 yrs). Although other studies have reported

TABLE 1 - *Place of residence, degree of education, employment and serology in Kosovo pregnant women.*

	Total population (No. 334)	*IgG+ (No. 97) 29,4%	IgM+ and avidity+ (No. 4/97) 4,1%
Place of residence*			
Urban	92	22 ns	3 ns
Rural	242	75 ns	1 ns
Degree of Education*			
≤8 years	106	26 ns	2 ns
≥8 years	228	71 ns	2 ns
Employment**			
Housewives	108	31 ns	3[§]
Unemployed	194	57 ns	0
Nurse	2	0 ns	0
Teacher	5	1 ns	0
Trainer	6	4 ns	1 ns
Student	2	0 ns	0
Private employment	2	0 ns	0
Not determined	15	4 ns	0

*Chi-square=0.68, p=0.40, distribution not significant; **t =1.1266, p=0.2788, distribution not significant for p<0.05; [§]p=0.000 distribution significant for p<0.05.

TABLE 2 - *T. gondii antibodies seroprevalence according to age in Kosovo pregnant women.*

Age groups (yrs)*	Total (334 pts)	IgG+ (97 pts)	IgM+ (4 pts)
17-28	150	38 (25,3%)	4 (2,7%)**
29-38	158	48 (30,4%)	0
39-46	22	11 (50,0%)	0

*Chi-square=2.830, p=0.243, distribution not significant; **Chi-square=4.86, p=0.0275 (prob. 5%) significant.

a significant variation of *Toxoplasma gondii* seropositivity according to age, when analyzing our data with a contingency table, the *p* value was not significant (p=0.243, chi-square =2.830 with 2 GL) [Nowakowska *et al*, 2006; Berger *et al*, 2009]. However, IgM positivity (2.6%) was present in the younger age group (17-28 yrs) as expected, with a statistical significance of p=0.0275.

DISCUSSION

Toxoplasma gondii, an obligate intracellular coccidian, can parasitize either humans or a wide range of other vertebrate species. Human infection is usually due to consumption of raw or poorly cooked meat containing bradyzoites, or ingestion of oocysts from cat faeces in soil, water or food; 90% of these cases are asymptomatic. It is estimated that 20-90% of adults throughout the world have come into contact with the parasite during their lifetime, [Galvan-Ramirez *et al.*, 1998] depending on the sanitary conditions of the country considered. In fact, *Toxoplasma* infection is related to several factors including socioeconomic level, nutritional habits, age, and rural or urban setting (Spalding *et al.*, 2005).

The diagnosis of a recently acquired primary *Toxoplasma* infection is difficult because the IgM antibodies (typical marker for recent infection) developed during toxoplasmosis may persist for many months and even years. Measuring the avidity-specific IgG has been demonstrated to be particularly useful for this purpose. Actually, the initial IgG antibody response to infection is characterized by antibodies with a low avidity in which binding to the specific antigen sites is easily dissociated (Zotti *et al.*, 2004; Montoya *et al.*, 2004; Tanyuksel *et al.*, 2004; Iqbal *et al.*, 2007).

In a survey conducted on 496 Albanian pregnant women (Maggi *et al.*, 2009), a 48.6% IgG seroprevalence was observed and an 1.2% IgM prevalence was found among IgG-positive women, 0.8% of whom showed low avidity. The rate of

IgG seroprevalence found among Kosovar pregnant women (29.4%) was lower than that observed in the rest of Europe. Conversely, the percentage of recent infections was higher than that expected (4.1%), indicating a low local circulation of the parasite in the past. In particular, housewives seem more exposed to the risk of recent infection.

The interpretation of these apparently contradictive data is not simple because the risk of newly acquired infections in a population of pregnant women depends both on the rate of infection in that specific geographic area and on the number of people previously non-infected; therefore changes in the incidence of acute infection over time within the same community might occur (De Pascale *et al.*, 2008). We hypothesize that our findings could be the expression of a recent change in the hygienic conditions due to the disastrous effects of a civil war (with possible circulation of uncontrolled contaminated food and/or improperly purified water), in a region previously characterized by a low prevalence of this disease. This hypothesis is also reinforced by the fact that housewives are a high risk group for acute infection as they are generally more exposed to contact with uncooked food and contaminated soil. In Kosovo, the social organization of the family in rural areas, such as Albania, dictates that housewives are also responsible for the care of the fields and cattle. However, other factors involved are more difficult to evaluate, such as the frequency of cats, the prevalence of Toxoplasma gondii in cats, meat production practices, and changes in water- and soil-related usages (Montoya *et al.*, 2004).

Our study presents some limitations mainly due to the fact that these data derive from a retrospective evaluation of pregnant women selected for various other purposes. For this reason important data, such as possible risk factors for *Toxoplasma* infection (consumption of uncooked meat, contact with cats or other animals…) are not available. Nevertheless, the hypothesis of a recent epidemic of this disease in Kosovo is consistent with our results. Undoubtedly, a more specific evaluation on a more extensive population is warranted. Moreover, the improvement of a disease-specific surveillance system in Kosovo is also advisable because the low prevalence of antitoxoplasma IgG antibodies among women of child-bearing age suggest a possible high risk of maternal toxoplasmosis infection acquired during pregnancy leading to a greater risk of fetal damage. Thus, in Kosovo, a health education program for all pregnant women together with serological testing for those exposed to risk factors for infection should be established as an epidemiological support and financially sustainable alternative.

REFERENCES

ALLAIN J.P., PALMER C.R., PEARSON G. (1998). Epidemiological study of latent and recent infection by *Toxoplasma gondii* in pregnant women from regional population in the UK. *J Infect*. **36**, 189-196.

BERGER F., GOULET V., LE STRAT Y., DESENCLOS J.C. (2009). Toxoplasmosis among pregnant women in France: risk factors and change of prevalence between 1995 and 2003. *Rev Epidemiol Sante Publique*. 57, 241-248.

BILEFSKY D. (2008). Kosovo declares its independence from Serbia. *The New York Times*.

BOBIC B., JEVREMOVIC I., MARINKOVIC J., SIBALIC D., DJURKOVIC-DJARKOVIC O. (1998). Risk factors for *Toxoplasma* infection in a reproductive age female population in the area of Belgrade, *Yugoslavia*. *Eur J Epidemiol*. **14**, 605-610.

BOBIC B., NIKOLIC A., DJURKOVIC-DJARKOVIC O. (2003). Identification of risk factors for infection with *Toxoplasma gondii* in Serbia as a basis of a program for prevention of congenital toxoplasmosis. *Srp Arh Celok Lek*. **131**, 162-167.

BOWIE W.R., KING A.S., WERKER D.H., ISAAC-RENTON J.L., BELL A., ENG S.B., MARION S.A. (1997). *Toxoplasma* Investigation Team. Outbreak of toxoplasmosis associated with municipal drinking water. *Lancet*. **350**, 173-177.

DE PASCALE M., AGRIPPI C., BELVISI L., CAGNIN D., CERULLI T., CLERICI P., MIRRI P., MANCO M.T., CAVALLAI S., VIGANO F.F. (2008). Revision of the positive predictive value of IgM anti-*Toxoplasma* antibodies a san index of the recent infection. *New Microbiol*. **31**, 105-111.

GALVAN-RAMIREZ M.L., GUILLEN-VARGAS C., SAAVEDRA-DURAN R., ISLOS-RODRIGUEZ A. (1998). Analysis of

Toxoplasma gondii antigens with sera from toxo-plasmosis patients. *Revista da Sociedade Brasiliera de Medicina Tropical.* **31**, 271-277.

GILBERT R. (1999). Epidemiology of infection in pregnant women. In: *Petersen E. & Amboise-thomas P., eds Congenital toxoplasmosis: scientific background, clinical management and control. Paris: Springer-Verlag France*.

HORION M., THOUMSIN H., SENTERRE J., LAMBOTTE R. (1990). 20 years of screening for toxoplasmosis in pregnant women. The Liege experience in 20,000 pregnancies. *Rev Med Liege.* **45**, 492-497.

IQBAL J., KHALID N. (2007). Detection of acute Toxoplasma gondii infection in early pregnancy by Igg avidity and PCR analysis. *J Med Microbiol.* **56**, 1495-1499.

JENUM P.A., KAPPERUD G., STRY PEDERSEN B., MELBY K.K., ESKILD A., ENG J. (1998). Prevalence of *Toxoplasma gondii* specific immunoglobulin G antibodies among pregnant women in Norway. *Epidemiol Infect.* **120**, 87-92.

LEBECH M., ANDERSEN O., CHRISTENSEN N.C., HERTEL J., NIELSEN H.E., PEITERSEN B., RECHNITZER C., LARSEN S.O., NORGAARD-PETERSEN E. (1999). Feasibility of neonatal screening for *Toxoplasma* infection in the absence of prenatal treatment. *Lancet.* **353**, 1834-1837.

MAGGI P., VOLPE A., CARITO V., SCHINAIA N., BINO S., BASHO M., DENTICO P. (2009). Surveillance of Toxoplasmosis in pregnant women in Albania. *New Microbiol.* **32**, 89-92.

MCCABE R.E., REMINGTON J.S. (1988). Toxoplasmosis: the time has come. *The New England Journal of Medicine.* **318**, 313-315.

MONTOYA J.G., HUFFMAN H.B., REMINGTON J.S. (2004). Evaluation of the immunoglobulin G avidity test for diagnosis of toxoplasmic lymphadenopathy. *J Clin Microbiol.* 42, 4627-4631.

NOWAKOWSKA D, STRAY-PEDERSEN B, SPIEWAK E, SOBALA W, MALAFIEJ E, WILCZYNSKI I. (2006). Prevalence and estimated incidence of Toxoplasma infection among pregnant women in Poland: a decreasing trend in the younger population. *Clin Microbiol. Infect.* **12**, 913-917.

QUAGLIO G., RAMANDANI N., PATTARO C., CAMI A., DENTICO P., VOLPE A., PELLIZZER G., BERISHA A., SMACCHIA C., FIGLIOMENI M., SCHINAIA N., REZZA G., PUTOTO G. (2008). Prevalence and risk factors for viral hepatitis in the Kosovarian population: implications for health policy. *J Med Virol.* 80, 833-840.

SPALDING S.M., AMENDOEIRA M.R.R., KLEIN C.H., RIBEIRO L.C. (2005). Serological screening and toxoplasmosis exposure factors among pregnant women in South of Brazil. *Rev Soc Bras Med Trop.* 38, 173-177.

TANYUKSEL M., GUNEY C., ARAZ E., SARACLI M.A., DOGANCI L. (2004). Performance of the immunoglobulin G avidity and enzyme Immunoassay IgG/IgM screening tests for differentiation of the clinical spectrum to toxoplasmosis. *J Microbiol.* **42,** 211-215.

TIZARD J.R., CHAUHAN S.S., LAI C.H. (1977). The prevalence and epidemiology of toxoplasmosis in Ontario. *J Hyg (Lond)* **78**, 275-282.

WILLIAMS K.A.B., SCOTT J.M., MACFARLANE D.E., WILLIAMS J.M., ELLASJONES T.F., WILLIAMS H. (1981). Congenital toxoplasmosis: a prospective survey in the West of Scotland. *J Infect.* **3**, 219-229.

WONG S.Y., REMINGTON J.S. (1994). Toxoplasmosis in pregnancy**.** *Clin Inf Dis* **18**, 853-862.

WORLD BANK. KOSOVO POVERTY ASSESSMENT (2005). Promoting opportunity, security, and participation for all. Poverty reduction and economic management unit. Europe and Central Asia Region. *Report n. 32378-XK*

ZOTTI C., CHARRIER L., GIACOMUZZI M., MOIRAGHI RUGGENINI A., MOMBRÒ M., FABRIS C., MAROCCHETTI P., ALFIERI R., LETO R., RENZI N., MILANO R., LIEVE M.A., COLOZZA M., ZANELLA D., ANTONA G., MASCHERO M.C., TOSETTI F., BIGLIETTI D., NICOLETTA T., DE RENZI G., TINIVELLA F., DONATI M., FERRINI A., CROTTI G., COUCOURDE L., GUAZZETTI G.C., GERA A., MALABAILA A., DE NATALE C., RABOZZI M.L., GINARDI C., BUZZONE T., CANEPA C., FRUTTERO M., MASTRACCHIO G., VALLE S., TOPPINO M., FORNO N., BELLINGERI P., CARACCIO W., LAZZARA C., DE CAROLI V., PEDRAZZI E., GOMELLA S., Collaborative Group (2004). Use of IgG avidity test in case definitions of toxoplasmosis pregnancy. *New Microbiol.* **27**, 17-20.