ORIGINAL ARTICLE 10.1111/j.1469-0691.2007.01771.x

New and old risk-factors for *Toxoplasma gondii* infection: prospective cross-sectional study among military personnel in the Czech Republic

P. Kolbekova[1], E. Kourbatova[2], M. Novotna[1], P. Kodym[3] and J. Flegr[1]

[1]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic, [2]Division of Tuberculosis and Lung Diseases, Samara State Medical University, Samara, Russian Federation and [3]National Reference Laboratory for Toxoplasmosis, National Institute of Public Health, Prague, Czech Republic

ABSTRACT

The aims of this study were to evaluate seroprevalence and the importance of various risk-factors for *Toxoplasma* infection in the Czech Republic. A prospective cross-sectional survey was conducted among military personnel in Prague. Consenting subjects (n = 3250) completed a questionnaire concerning demographics and risk-factors, and blood samples were taken to determine anti-*Toxoplasma* antibody titres according to complement fixation and ELISA IgG and IgM tests. The seroprevalence of toxoplasmosis was 23%. In multivariate analysis, independent predictors of *Toxoplasma* seropositivity were age (OR 1.03 / year), consumption of raw meat (OR 1.35), owning a cat (OR 1.25), owning rabbits (OR 1.47), childhood residence in a town with a population of <10 000 inhabitants (OR 1.63) vs. location of the childhood residence in a town with population of >100 000 inhabitants, and blood group type A (OR 1.28), B (OR 1.33) or AB (OR 1.43) vs. O. These results suggested that horizontal toxoplasmosis transmission in the Czech Republic may occur through consumption of raw meat, contact with cat faeces and farming.

Keywords Cats, Czech Republic, risk-factors, seroprevalence, toxoplasmosis, transmission

Original Submission: 10 January 2007; **Revised Submission:** 23 March 2007; **Accepted:** 23 April 2007

Clin Microbiol Infect 2007; **13**: 1012–1017

INTRODUCTION

Toxoplasmosis is one of the most common parasitic zoonoses worldwide. Horizontal transmission of *Toxoplasma gondii* among humans occurs primarily in one of two ways, namely, ingestion of infectious oocysts from the environment (e.g., subsequent to gardening, changing a cat litter box or eating unwashed vegetables), or ingestion of tissue cysts, present in raw or undercooked meat. Vertical transmission of newly acquired *T. gondii* infection can occur from a pregnant woman to the foetus via the placenta. Toxoplasmosis acquired post-natally can cause severe encephalitis via acute infection or reactivation of latent infection in immunocompromised patients [1]. However, immunocompetent individuals with latent toxoplasmosis do not have distinctive clinical signs, and the only evidence of infection is the activation of the immune system against *T. gondii* antigens.

The estimated seroprevalence of toxoplasmosis in human populations worldwide ranges from 9 to 78% [2,3]. The major routes of transmission vary among different countries, and may depend on cultural and eating habits [4]. An understanding of the major routes of horizontal transmission to humans, and the most likely sources of infection in a given population, is important for the development of effective public health measures for the prevention of toxoplasmosis infection in particular risk groups, including non-immune (Toxo-free) pregnant females and immunocompromised patients, and especially those individuals with advanced human immunodeficiency virus disease. The purpose of the present study was to evaluate seroprevalence and to study the

Corresponding author and reprint requests: J. Flegr, Department of Parasitology, Charles University, Vinicna 7, Prague 12844, Czech Republic
E-mail: flegr@cesnet.cz

importance of various risk-factors for toxoplasmosis in the population of the Czech Republic.

MATERIALS AND METHODS

Study population and data collection

A prospective cross-sectional survey was conducted among Czech military personnel between 1 April 2000 and 30 September 2004 at the Department of Psychology of the Central Military Hospital, Prague, Czech Republic. Military service in the Czech Republic was compulsory before the year 2005; thus, the recruits provided a representative sample of the Czech male population. All study participants provided written informed consent, and the study was approved by the Ethical Committee of the Faculty of Science of Charles University, Prague.

The study population consisted of two sub-populations (sets): the first set (n = 2616) comprised recruits for compulsory military service; the second set (n = 674) comprised professional full-time military personnel, including the staff of international military missions, the Implementation Force (IFOR) and the Stabilisation Force (SFOR).

Demographical characteristics and information concerning probable risk-factors for toxoplasmosis infection were recorded at study enrolment using a standardised questionnaire. The questionnaire included questions concerning demographical characteristics (age, gender, date of birth, education, marital status, siblings, type of military service), population size of current and childhood residence (city/village), eating habits (i.e., eating or tasting raw meat), and current or past ownership of animals (cats, dogs and rabbits) in the participant's family. Blood samples for toxoplasmosis testing and for determination of blood group type were taken from all consenting participants.

Laboratory methods

All serum samples were tested for toxoplasmosis at the National Reference Laboratory, National Institute of Public Health, Prague, using a complement fixation test (CFT) (SEVAPAHARMA, Prague, Czech Republic) and an IgG ELISA (SEVAPAHARMA). Samples with high IgG levels were also tested with an IgM ELISA (TEST-LINE, Brno, Czech Republic). Latent toxoplasmosis was diagnosed on the basis of a positive CFT (titre ≥8) and a positive IgG ELISA test (according to the manufacturer's recommendations), combined with a negative or equivocal IgM ELISA test [5]. Suspected acute toxoplasmosis was diagnosed on the basis of a positive IgM ELISA test (positivity index >1.1). Subjects with discordant CFT and ELISA results were excluded from the analysis of risk-factors for *Toxoplasma* infection. In addition, ABO and Rh blood types were determined for all serum samples using a microplate method (KlinLab Ltd, Prague, Czech Republic).

Data analysis

The data were analysed using a case-control study design to identify factors associated with the risk of having *Toxoplasma* infection. Cases were defined as subjects with *Toxoplasma* infection (positive in both the CFT and ELISA tests). Controls were defined as subjects who did not have *Toxoplasma* infection (negative in both the CFT and ELISA tests). Data management and statistical analyses were performed using SAS software, v.8.2 (SAS Institute Inc., Cary, NC, USA). Potential risk-factors were assessed initially by univariate analysis. Categorical data were compared using the Pearson chi-square test, or Fisher's exact test when the sample size was ≤5. Trends were assessed using the Cochran–Armitage trend test. Continuous variables were compared using a paired t-test. Mantel–Haenszel ORs and corresponding 95% CIs were calculated for dichotomous variables. Variables associated with an increased risk of having *Toxoplasma* infection in univariate analyses (i.e., variables with $p < 0.1$), potential effect modifiers and confounders were entered in an unconditional multivariate logistic regression model. Possible two-way and higher interactions among the main effect variables were examined (no interaction was observed). Confounding was tested and the final model was derived by incorporating a hierarchical backward elimination approach [6]. Co-linearity and goodness-of-fit of the final logistic regression model were assessed, with $p \leq 0.05$ being defined as statistically significant.

RESULTS

In total, 3290 representatives from the Czech military population were recruited to the study and were tested using CFT and ELISA. Forty (1.2%) subjects with discordant CFT and ELISA results were excluded from the analysis of risk-factors for *Toxoplasma* infection (29 (0.9%) were positive by ELISA and negative by CFT; 11 (0.3%) were positive by CFT and negative by ELISA). The median age of the remaining 3250 study participants was 20 years (range 17–60 years); all were male.

The overall seroprevalence of *Toxoplasma* infection was 23%. In individuals with *Toxoplasma* infection, IgG antibodies were found at concentrations of 18–11 982 optical units (median 133), and in CFT at titres of 8–1024 (median 16). One (0.03%) individual had suspected acute toxoplasmosis infection (ELISA IgM positivity index 2.5).

The highest seroprevalence was detected in the groups aged 30–34, 35–39 and ≥45 years (35%, 30% and 29%, respectively) (Fig. 1). The overall trend for increasing seroprevalence of *Toxoplasma* infection was statistically significant ($p < 0.001$), while the decreasing trend for individuals aged ≥35 years was not significant (p 0.31). The seroprevalence of *Toxoplasma* infection among individuals with different blood groups was 20% (213/1052) for type O, 24% (324/1328) for type A, 25% (150/600) for type B, and 26% (66/254) for type AB.

The detailed results of the univariate analysis are shown in Table S1 (see Supplementary material). The risk of *Toxoplasma* infection increased significantly with age, with a significantly ele-

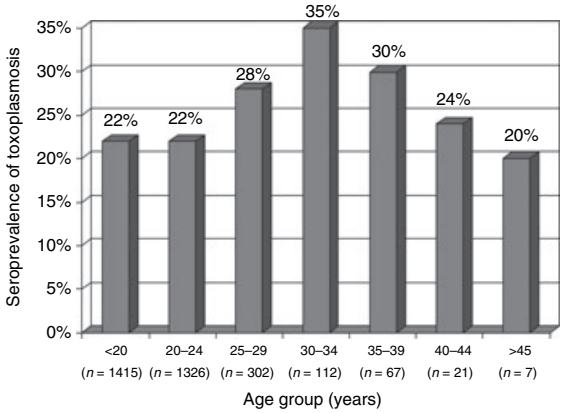

Fig. 1. Seroprevalence of *Toxoplasma* infection in different age groups.

vated risk in the groups aged 25–29 years and 30–34 years, as compared to the group aged <20 years.

Current residence in a town/village with <10 000 inhabitants or 10 000–50 000 inhabitants (compared with living in a city with >100 000 inhabitants), and childhood residence in a town/village with <10 000 inhabitants (compared with location of the childhood residence in a city with >100 000 inhabitants), were associated significantly with seropositivity following univariate analysis. Married status (compared with single status) and being a professional member of the military (compared with being a new recruit in regular compulsory service) were also significant risk-factors following univariate analysis.

Toxoplasma infection was associated significantly with eating or tasting raw meat, owning a cat, owning rabbits, and owning a dog. Blood groups A, B or AB, compared with blood group O, were also associated significantly with *Toxoplasma* infection.

In multivariate analysis (Table S2; see Supplementary material), independent predictors of *Toxoplasma* seropositivity were age (OR 1.03/year, 95% CI 1.02–1.05), eating or tasting raw meat (OR 1.35, 95% CI 1.14–1.60), owning a cat (OR 1.25, 95% CI 1.04–1.50) or rabbits (OR 1.47, 95% CI 1.22–1.77), childhood residence in a town/village with a population of <10 000 inhabitants (OR 1.63, 95% CI 1.25–2.12), and having blood group A (OR 1.28, 95% CI 1.05–1.56), B (OR 1.33, 95% CI 1.04–1.69) or AB (OR 1.43, 95% CI 1.03–1.97). When the multivariate analysis was repeated with variable current residence population size instead of childhood residence population size, the final model had the same risk-factors, and current residence population size was a significant predictor of toxoplasmosis (results not shown).

DISCUSSION

The overall seroprevalence of *Toxoplasma* in the present study was 23%, which was similar to that found in previous studies in the Czech Republic, where toxoplasmosis seroprevalence was reported to be 26.3% in the male population [7], 25–33% among blood donors [8,9], and 29–37% among pregnant women [10]. In comparison, the reported *Toxoplasma* seroprevalence in other European countries ranges from 9.1% among pregnant women in the UK [11] to 71% in France [3] and 77% in Yugoslavia [12]. A high *Toxoplasma* seroprevalence (61–78%) has also been reported for countries in Central and South America [13,14] and in sub-Saharan Africa [15]. In the USA, the overall national seroprevalence has been estimated as 22.5% [2]. A large study conducted in the USA in 1989 reported a prevalence of 9.5% among military recruits, with 80% of participants being aged 17–20 years [16].

The present study found that the seroprevalence of toxoplasmosis increased with age, possibly because of a higher probability of contact with oocysts of *Toxoplasma* during professional activity, gardening, etc. The age trends could also be explained by a cohort effect [2,17], as the risk of *Toxoplasma* infection may have been higher in the past because the use of frozen meat was less common and animal rearing practices have subsequently improved [2]. Similar age-related increases in *Toxoplasma* seroprevalence have been reported in numerous studies of various populations worldwide [2,7,11,12,17–19]. Eating raw or undercooked meat has also been demonstrated previously to be a risk-factor for *Toxoplasma* infection [12,20–23], with only a few studies failing to identify such an association [11,18]. A study of pregnant women in six large European cities (Naples, Lausanne, Copenhagen, Oslo, Brussels and Milan) found that 30–63% of acute *Toxoplasma* infections in the different study centres were attributed to consumption of undercooked or cured meat products [20].

In meat-producing animals, tissue cysts of *Toxoplasma* are observed most frequently in the

tissues of infected sheep, goats, pigs and, less frequently, poultry and rabbits [4], although the prevalence of tissue cysts is very high in rabbits in the Czech Republic [24]. The types of meat products associated with the transmission of *Toxoplasma* are different in human populations with varying eating habits. Thus, a European multicentre study [20] found that the risk-factors most strongly predictive of acute *Toxoplasma* infection in pregnant women were consumption of undercooked lamb, beef or game, while a study conducted in Norway [21] found that *Toxoplasma* maternal seropositivity was associated with eating raw or undercooked mutton and pork, and a study conducted among women in California in the USA found that a major infection source was consumption of rare/medium cooked beef [22]. The reported seroprevalence of *Toxoplasma* infection among meat-producing animals in the Czech Republic in 1979–2000 ranged from 53% in rabbits [24] to 21–61% in goats [25], 13–55% in sheep [25], <1–35% in pigs [26], and 2–22% in cattle and buffalo [27]. On the basis of these findings, it seems that the most likely meat sources of *Toxoplasma* infection in the Czech Republic could be rabbits, sheep and goats.

Owning rabbits was found to be associated independently with *Toxoplasma* seropositivity. Keeping rabbits for meat is quite common in the Czech Republic, and 33% of the families of the participants in the present study had owned rabbits. Transmission of *Toxoplasma* from rabbits to humans could be realised by tasting or eating undercooked rabbit meat containing *Toxoplasma* cysts, or through skin injuries during skinning the rabbits and preparing meals. It is also possible for the hay used by rabbits to be contaminated by oocysts from infected cat faeces of stray or pet cats that roam on farms and frequently visit the haylofts. *Toxoplasma* infection could be transmitted to humans by ingestion or inhalation of oocysts from the environment during caring for rabbits or farming. Owning a cat has been shown to be a risk-factor for human toxoplasmosis in several previous studies [17,21,22], although other studies have demonstrated that contact with cats is not a risk-factor for *Toxoplasma* infection in certain populations [12,18,20,28]. Svobodova *et al*. [29] estimated that the seroprevalence of *Toxoplasma* among cats in the Czech Republic was 59%. Epidemiologically, there are numerous stray cats that live on farms and are allowed to roam freely; these may contaminate the environment with oocysts which can infect livestock that will later be slaughtered for human consumption [4].

The present study showed an association between residence in a rural location and *Toxoplasma* infection; thus, in urban districts of the Czech Republic, the average prevalence reached 19.6%, compared with 35.3% in rural districts [7]. An association between rural location of the childhood residence and *Toxoplasma* infection has also been demonstrated in a study of pregnant women in the UK [11]. Contact with soil, gardening and soil-related occupations have been shown to be risk-factors for toxoplasmosis in a number of studies [2,3,17,22]. The association between residence in a small town/village and having toxoplasmosis probably reflects more frequent contact with soil through gardening and farming in rural areas.

It is of interest that the study revealed an association between blood group type and *Toxoplasma* infection, with the lowest prevalence of *Toxoplasma* infection being among individuals with blood group O (20%), and the highest prevalence among individuals with blood group AB (26%). A study among blood donors in Russia has reported similar findings, with *Toxoplasma* seroprevalence being twice as high among subjects with blood group AB than among subjects with blood group O (54% vs. 27%, respectively) [30]. It is known that natural resistance to many infectious diseases may depend, to a certain extent, on the blood group of an individual [31,32]. The A, B and O blood group phenotypes are determined by the presence or absence of A and/or B carbohydrate antigens on the surface of red blood cells [33]. This determines natural resistance in humans to many infectious disease agents that have cell surface antigens similar to the antigens of different blood group types. This mechanism may, in part, explain the higher susceptibility of individuals with blood type AB to several infectious diseases, since the blood of these individuals does not contain the corresponding natural antibodies.

The present study was subject to several limitations. The study population included relatively young and healthy men, and the findings may therefore differ from findings in other populations. Self-reported data obtained from questionnaires could potentially introduce a misclassification bias. Several factors reported by other studies to be risk-factors for toxoplasmosis

were not assessed, e.g., foreign birthplace [2,11,17,19], educational level [2], socio-economic status [2,14,34], consumption of drinking water other than bottled water [18], drinking unfiltered water [14], and eating unwashed raw vegetables or fruit [21]. Despite these limitations, the study provided good data concerning the seroprevalence of *Toxoplasma* infection in a population of military personnel, which was a fairly representative sample of the general population of healthy men in the Czech Republic.

Since the elimination of *Toxoplasma* parasites in animal sources is not feasible, public health measures directed towards the prevention of toxoplasmosis should be focused primarily on the prevention of transmission of *Toxoplasma* from known animal sources to humans. The most important recommendation for prevention of *Toxoplasma* infection is to avoid eating raw or incompletely cooked meat. Particular attention should be given to the handling and thorough cooking of rabbit meat, since the prevalence of *Toxoplasma* in rabbits is high in the Czech Republic. Further studies are necessary to explore the impact of host genetic factors on the acquisition of *Toxoplasma* infection.

ACKNOWLEDGEMENTS

This research was supported by Grant Agency of Czech Republic (406/07/0360) and the Czech Ministry of Education (grant 0021620828).

SUPPLEMENTARY MATERIAL

The following supplementary material for this article is available online at http://www.blackwell-synergy.com:

Table S1. Univariate analysis of risk-factors for *Toxoplasma* infection among military personnel in the Czech Republic.

Table S2. Multivariate analysis of risk-factors for *Toxoplasma* infection among military personnel in the Czech Republic.

REFERENCES

1. Hill D, Dubey JP. *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clin Microbiol Infect* 2002; **8**: 634–640.
2. Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T, McAuley JB. *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. *Am J Epidemiol* 2001; **154**: 357–365.
3. Jeannel D, Niel G, Costagliola D, Danis M, Traore BM, Gentilini M. Epidemiology of toxoplasmosis among pregnant women in the Paris area. *Int J Epidemiol* 1988; **17**: 595–602.
4. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol* 2000; **30**: 1217–1258.
5. Valkoun A, Stefanik M, Nadvornik V, Kodym P. Diagnosis of acquired toxoplasmosis using simultaneous determination of specific immunoglobulins M, A and E. *Epidemiol Mikrobiol Imunol* 1995; **44**: 107–110.
6. Kleinbaum DG, Klein M. *Logistic regression: a self-learning text*, 2nd edn. New York: Springer-Verlag, 2002.
7. Kodym P, Maly M, Svandova E *et al*. *Toxoplasma* in the Czech Republic 1923–1999: first case to widespread outbreak. *Int J Parasitol* 2001; **31**: 125–132.
8. Hejlicek K, Literak I, Chalupa B. Occurrence of *Toxoplasma gondii* antibodies in blood donors 1980–1990. *Cesk Epidemiol Mikrobiol Imunol* 1992; **42**: 135–140.
9. Svobodova V, Literak I. Prevalence of IgM and IgG antibodies to *Toxoplasma gondii* in blood donors in the Czech Republic. *Eur J Epidemiol* 1998; **14**: 803–805.
10. Hejlicek K, Literak I, Vostalova E, Kresnicka J. *Toxoplasma gondii* antibodies in pregnant women in the Ceske Budejovice District. *Epidemiol Mikrobiol Imunol* 1999; **48**: 102–105.
11. Nash JQ, Chissel S, Jones J, Warburton F, Verlander NQ. Risk factors for toxoplasmosis in pregnant women in Kent, United Kingdom. *Epidemiol Infect* 2005; **133**: 475–483.
12. Bobic B, Jevremovic I, Marinkovic J, Sibalic D, Djurkovic-Djakovic O. Risk factors for *Toxoplasma* infection in a reproductive age female population in the area of Belgrade, Yugoslavia. *Eur J Epidemiol* 1998; **14**: 605–610.
13. Frenkel JK, Ruiz A. Human toxoplasmosis and cat contact in Costa Rica. *Am J Trop Med Hyg* 1980; **29**: 1167–1180.
14. Bahia-Oliveira LM, Jones JL, Azevedo-Silva J, Alves CC, Orefice F, Addiss DG. Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro state, Brazil. *Emerg Infect Dis* 2003; **9**: 55–62.
15. Onadeko MO, Joynson DH, Payne RA. The prevalence of *Toxoplasma* infection among pregnant women in Ibadan, Nigeria. *J Trop Med Hyg* 1992; **95**: 143–145.
16. Smith KL, Wilson M, Hightower AW *et al*. Prevalence of *Toxoplasma gondii* antibodies in US military recruits in 1989: comparison with data published in 1965. *Clin Infect Dis* 1996; **23**: 1182–1183.
17. Kortbeek LM, De Melker HE, Veldhuijzen IK, Conyn-Van Spaendonck MA. Population-based *Toxoplasma* seroprevalence study in The Netherlands. *Epidemiol Infect* 2004; **132**: 839–845.
18. Ertug S, Okyay P, Turkmen M, Yuksel H. Seroprevalence and risk factors for toxoplasma infection among pregnant women in Aydin province, Turkey. *BMC Public Health* 2005; **5**: 66.
19. Falusi O, French AL, Seaberg EC *et al*. Prevalence and predictors of *Toxoplasma* seropositivity in women with and at risk for human immunodeficiency virus infection. *Clin Infect Dis* 2002; **35**: 1414–1417.
20. Cook AJ, Gilbert RE, Buffolano W *et al*. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. *BMJ* 2000; **321**: 142–147.

21. Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, Eskild A, Eng J. Risk factors for *Toxoplasma gondii* infection in pregnancy. Results of a prospective case-control study in Norway. *Am J Epidemiol* 1996; **144**: 405–412.
22. MacKnight KT, Robinson HW. Epidemiologic studies on human and feline toxoplasmosis. *J Hyg Epidemiol Microbiol Immunol* 1992; **36**: 37–47.
23. Buffolano W, Gilbert RE, Holland FJ, Fratta D, Palumbo F, Ades AE. Risk factors for recent toxoplasma infection in pregnant women in Naples. *Epidemiol Infect* 1996; **116**: 347–351.
24. Hejlicek K, Literak I. Prevalence of toxoplasmosis in rabbits in South Bohemia. *Acta Vet Brno* 1994; **63**: 145–150.
25. Hejlicek K, Literak I. Incidence and prevalence of toxoplasmosis among sheep and goat in southern and western Bohemia. *Acta Vet Brno* 1994; **63**: 151–159.
26. Hejlicek K, Literak I. Prevalence of toxoplasmosis in pigs in the region of South Bohemia. *Acta Vet Brno* 1993; **62**: 159–166.
27. Hejlicek K, Literak I. Occurrence of toxoplasmosis and its prevalence in cattle in the south Bohemia region. *Acta Vet Brno* 1992; **61**: 195–206.
28. Wallace MR, Rossetti RJ, Olson PE. Cats and toxoplasmosis risk in HIV-infected adults. *JAMA* 1993; **269**: 76–77.
29. Svobodova V, Knotek Z, Svoboda M. Prevalence of IgG and IgM antibodies specific to *Toxoplasma gondii* in cats. *Vet Parasitol* 1998; **80**: 173–176.
30. Zhiburt EB, Ionova AI, Danil'chenko VV, Serebrianaia NB, Bel'gesov NV, Trofimenko EV. The spread of antibodies to cytomegalovirus and *Toxoplasma* among donors of blood components. *Zh Mikrobiol Epidemiol Immunobiol* 1997; **1**: 59–61.
31. Lell B, May J, Schmidt-Ott RJ *et al.* The role of red blood cell polymorphisms in resistance and susceptibility to malaria. *Clin Infect Dis* 1999; **28**: 794–799.
32. Fisher PR, Boone P. Short report: severe malaria associated with blood group. *Am J Trop Med Hyg* 1998; **58**: 122–123.
33. Hakomori S. Antigen structure and genetic basis of histoblood groups A, B and O: their changes associated with human cancer. *Biochim Biophys Acta* 1999; **1473**: 247–266.
34. Guerra GC, Fernandez SJ. Seroprevalence of *Toxoplasma gondii* in pregnant women. *Aten Primaria* 1995; **16**: 151–153.