ORIGINAL ARTICLE 10.1111/j.1469-0691.2005.01193.x

# Seroprevalence of *Toxoplasma gondii* in northern Greece during the last 20 years

*E. Diza, F. Frantzidou, E. Souliou, M. Arvanitidou, G. Gioula and A. Antoniadis*

First Department of Microbiology, School of Medicine, Aristotle University of Thessaloniki, Greece

## ABSTRACT

The seroprevalence of *Toxoplasma gondii* in the northern Greek population was determined in 1984, 1994 and 2004, and changes during this period were investigated. In total, 1014, 812 and 958 sera from individuals aged 1 day to 70 years were examined in 1984, 1994 and 2004, respectively, for IgG and IgM anti-*Toxoplasma* antibodies with the standard immunofluorescence assay (IFA) and microparticle enzyme immunoassay (MEIA). In individuals positive for IgM-specific antibodies, primary infection with *Toxoplasma* was diagnosed on the basis of the *Toxoplasma* serological profile (IFA, MEIA, conventional IgM and IgA ELISAs, immunosorbent agglutination assay and IgG avidity test). The prevalence of IgG-specific antibodies in the general population was 37%, 29.9% and 24.1% in 1984, 1994 and 2004, respectively, and was 35.6%, 25.6% and 20%, respectively, in women of reproductive age (15–39 years). The incidence of *Toxoplasma* infection, based on cases of primary infection and the annual seroconversion rate for the general population, was estimated to be 1.25% and 1.1% in 1984, 1.05% and 0.93% in 1994, and 0.85% and 0.8% in 2004. The significant decline in prevalence, and the shift towards an older age group, observed during this period could be explained by the improved socio-economic situation. The high (80%) proportion of women of reproductive age susceptible to *Toxoplasma* infection, with an estimated 90–200 neonates infected *in utero* annually, seems to present a potential risk to public health. Education of the public and prophylactic measures may become increasingly important.

**Keywords** Immunofluorescence assay, incidence, northern Greece, prevalence, seroprevalence, *Toxoplasma gondii*

**Original Submission:** 24 August 2004; **Revised Submission:** 27 December 2005; **Accepted:** 4 March 2005

*Clin Microbiol Infect 2005;* 11: 719–723

## INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*. Although the course of infection is generally benign, this organism can cause significant morbidity and mortality in the developing foetus and in immunocompromised individuals [1]. As an effective vaccine has not yet been developed, continuous and detailed epidemiological surveillance is required to estimate the risk of infection, especially in pregnant women, and the likelihood of reactivation in immunocompromised individuals.

The first seroepidemiological study of *T. gondii* in the regions of Macedonia and Thrace (northern Greece) was conducted in 1972, and revealed a prevalence rate of 43% [2]. The present study, performed in 2004, determined the prevalence and incidence of *Toxoplasma* infection in individuals aged 1 day to 70 years from the same area, as well as in the sub-population comprising women of reproductive age. The results obtained were compared with those from similar surveys performed in 1984 and 1994 [3,4].

Corresponding author and reprint requests: E. Diza, First Department of Microbiology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki 541 24, Greece
E-mail: eudoxia@med.auth.gr

## MATERIALS AND METHODS

### Study population

The individuals examined in 1984, 1994 and 2004 were selected randomly from the general population living in rural and urban areas of the 16 prefectures in northern Greece (population 2.8 million) [5]. The number of individuals tested from each prefecture was proportional to the population of the respective region. Serum samples were obtained from apparently healthy individuals, as follows: children who were participating in screening programmes for prevention of haemoglobulinopathies and for investigation of lipidaemic profiles; women tested before and during pregnancy; and adults who attended the hospital for an

annual medical check-up or for blood donation. In total, 1014 sera were tested in 1984 (470 males, 544 females), 812 in 1994 (390 males, 422 females), and 958 in 2004 (460 males, 498 females). All participants were grouped into ten age groups: 0–0.9, 1–4, 5–9, 10–14, 15–19, 20–29, 30–39, 40–49, 50–59 and 60–70 years. Women of reproductive age (15–39 years) were also studied as a separate group. The sera were stored at −70°C until required for analysis.

### Serological methods

All serum samples were examined for the presence of *Toxoplasma*-specific IgG and IgM antibodies separately. IgG antibodies were measured using an in-house immunofluorescence assay (IFA) [6], with results expressed in IU/mL, based on the WHO first standard serum 1967 [7]. Values ≥12 IU/mL were considered to be positive. In the case of equivocal results, the respective sera were also tested for specific IgG antibodies by microparticle enzyme immunoassay (IgG-MEIA; Abbott Laboratories, Chicago, IL, USA). IgM-specific antibodies were detected using IgM-MEIA (Abbott Laboratories).

IgM-positive sera were further tested by: (1) other IgM detection methods, including an in-house IFA assay (IgM-IFA; positive = ≥ 1:50 dilution), an immunosorbent agglutination assay (bioMérieux, Marcy l'Etoile, France), and a Western blotting test for specific IgM antibodies against the 30-kDa peptide (MarDx Diagnostics, Carlsbad, CA, USA); (2) a sensitive capture ELISA detecting IgA-specific antibodies (Bouty SpA, Sesto S. Giovanni, Italy; positive = ≥ 10 AU/mL); and (3) an assay measuring the avidity of IgG-specific antibodies (Bouty SpA), where an avidity of <15% indicates an acute primary infection during the last 2–3 months, 15–25% indicates a primary infection during the last 6 months, and >25% indicates an older infection.

Sera were tested by both IgG-IFA and IgM-IFA, or only by IgM-MEIA, at the time of collection. The additional methods for IgM, IgA and for determining the avidity of IgG antibodies were performed in 2004 for all IgM-positive sera.

### Diagnostic criteria for *Toxoplasma* primary infection

A positive IgM result has a low predictive value for identifying a primary infection with *Toxoplasma* [8,9]. A combination of serological assays can be used to determine the onset of infection with more certainty [10]. In the present study, a primary infection was considered to have occurred recently (i.e., during the previous 5–6 months) if there was a combination of high titres of IgG-IFA, positive IgM antibodies, and a low-avidity IgG antibody index (< 25%) in a single serum sample [8–12]. The incidence of primary infection with *Toxoplasma* was estimated from (1) the number of cases of primary infection/year, and (2) the annual seroconversion rate of the age-specific seroprevalence [13].

### Statistical methods

The chi-square test was used to compare differences in prevalence rates between age groups and to assess trends over time. A p value of $< 0.05$ was considered to be significant. Biostatistical analysis was performed using SPSS for Windows v. 10.0.1 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Immune status against *T. gondii*

A summary of all serological tests used and the diagnosis of immune status, based on the combined results, is shown in Table 1. The frequency of IgG-IFA anti-*Toxoplasma* antibodies in 1984, 1994 and 2004 is shown in Table 2. In all three years, seropositivity rose gradually with age; this rise was most rapid in 1984 and became slower in 1994 and in 2004. Thus, only a small proportion of children aged 1–4 and 5–9 years (3.2% and 7.4%, respectively) were positive in 2004, and the seroprevalence then rose gradually until the age of 49 years (33.3%). A significantly higher rate (50%) was found only in the group aged 50–59 years (p 0.019), with the highest rate (56.4%) in the group aged 60–70 years. No significant difference was found between men and women, except in 2004 for the group aged

**Table 1.** Determination of immune status for *Toxoplasma gondii* in the general population of northern Greece (1984, 1994 and 2004)

| | IgG-IFA | | | | | | | IgG avidity | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Year | Result (IU/mL) | No. positive sera | IgM-IFA-positive | IgM-MEIA-positive | IgM-ISAGA-positive | IgM-WB-positive | IgA-ELISA-positive | Result | No. sera | Immune status |
| 1984: | 12–800 | 374 | 9 | 9 | 9 | 2 | 3 | > 25% | 9 | Old infection |
| 1014 sera tested | 400–3200 | 4 | 2 | 4 | 4 | 4 | 3 | < 25% | 4 | Recent infection[a] |
| | < 12 | 634 | 0 | ND | ND | ND | ND | | | Negative |
| | < 12 | 2 | 2 | 1 | 0 | 0 | 0 | | | Negative |
| 1994: | 12–400 | 239 | 10 | 14 | 13 | 6 | 5 | > 25% | 14 | Old infection |
| 812 sera tested | 400–1600 | 3 | 2 | 3 | 3 | 3 | 3 | < 25% | 3 | Recent infection[a] |
| | < 12 | 567 | ND | 0 | ND | ND | ND | | | Negative |
| | < 12 | 3 | 1 | 3 | 1 | 0 | 0 | | | Negative |
| 2004: | 12–800 | 228 | 6 | 9 | 7 | 5 | 2 | > 25% | 9 | Old infection |
| 958 sera tested | 200–1600 | 3 | 3 | 3 | 3 | 3 | 3 | < 25% | 3 | Recent infection[a] |
| | < 12 | 723 | ND | 0 | ND | ND | ND | | | Negative |
| | < 12 | 4 | 2 | 4 | 2 | 0 | 0 | | | Negative |

[a]Primary infection acquired during the previous 6 months.
IFA, immunofluorescence assay; MEIA, microparticle enzyme immunoassay; ISAGA, immunosorbent agglutination assay; WB, Western blot; ND, not determined.