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

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**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% in 1984, 29.9% in 1994 and 24.1% in 2004, 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% in 1984, 1.05% in 1994, and 0.85% 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*

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**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].

**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: 0–9, 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)

<table>
<thead>
<tr>
<th>Year</th>
<th>IgG-IFA</th>
<th>IgM-IFA</th>
<th>IgM-MEIA</th>
<th>IgM-ISAGA</th>
<th>IgM-WB</th>
<th>IgM-ELISA</th>
<th>IgG avidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Result (IU/mL)</td>
<td>No. positive sera</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
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<tr>
<td>1984</td>
<td>12–800</td>
<td>374</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>&lt; 12</td>
<td>634</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1994</td>
<td>12–400</td>
<td>239</td>
<td>10</td>
<td>14</td>
<td>13</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>&lt; 12</td>
<td>567</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2004</td>
<td>12–800</td>
<td>228</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&lt; 12</td>
<td>723</td>
<td>3</td>
<td>3</td>
<td>3</td>
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<td>2</td>
</tr>
</tbody>
</table>

*pPrimary infection acquired during the previous 6 months.

IFA, immunofluorescence assay; MEIA, microparticle enzyme immunoassay; ISAGA, immunosorbent agglutination assay; WB, Western blot; ND, not determined.
60–70 years (43% in men vs. 56.4% in women; p 0.003). Overall, the seroprevalence rate in the general population in 2004 was 24.1%, which was significantly lower than the rates of 29.9% in 1994 (p 0.001) and 37% in 1984 (p 0.0059).

Incidence of primary infection with *Toxoplasma* in the general population

The two approaches used to calculate the incidence of primary *Toxoplasma* infection provided similar results (Table 3). Thus: (1) a primary infection was identified during the previous 6 months for four cases in 1984, three cases in 1994 and three cases in 2004, with estimated incidences of primary infection of 1.25, 1.05 and 0.83/100 seronegative individuals/year in 1984, 1994 and 2004, respectively; (2) the age-specific incidence of primary infection, estimated from the annual rate of seroconversion, increased annually by c. 1.1% in 1984, 0.93% in 1994 and 0.8% in 2004.

Table 3. Seroprevalence and incidence among population groups in northern Greece

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<tbody>
<tr>
<td>General population</td>
<td>37.3 29.8 24.1 1.25a</td>
<td>1.05a 0.83a 1.1a 0.93a 0.8a</td>
<td>0.69b 0.65b 0.5b 0.5b 0.4b</td>
<td>0.69b 0.65b 0.5b 0.5b 0.4b</td>
<td>35.6 25.6 20</td>
<td>0.69b 0.65b 0.5b 0.5b 0.4b</td>
<td>0.69b 0.65b 0.5b 0.5b 0.4b</td>
<td>0.69b 0.65b 0.5b 0.5b 0.4b</td>
<td></td>
<td></td>
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<tr>
<td>Women aged 15–39 years</td>
<td>35.6 25.6 20</td>
<td>0.69b 0.65b 0.5b 0.5b 0.4b</td>
<td>0.69b 0.65b 0.5b 0.5b 0.4b</td>
<td>0.69b 0.65b 0.5b 0.5b 0.4b</td>
<td>35.6 25.6 20</td>
<td>0.69b 0.65b 0.5b 0.5b 0.4b</td>
<td>0.69b 0.65b 0.5b 0.5b 0.4b</td>
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Prevalence and incidence of *Toxoplasma* infection in women of reproductive age

The prevalence of *Toxoplasma* infection in women of reproductive age (15–39 years) was 35.6% in 1984, 25.6% in 1994 and 20% in 2004 (Table 3). Based on the number of cases of primary infection, the incidence/pregnancy was estimated to be 0.69% in 1984, 0.67% in 1994 and 0.55% in 2004, while the annual seroconversion rate was 0.81% in 1984, 0.55% in 1994 and 0.48% in 2004. The mean incidence of primary infection/pregnancy was 0.51% in 2004.

DISCUSSION

The present study showed that 24.1% of healthy individuals in northern Greece, aged 1 day to 70 years, were positive for anti-*Toxoplasma* antibodies in 2004, and that 20% of women of reproductive age in the same population were seropositive. These seropositivity rates are significantly lower than rates reported in central European countries, but higher than those in the UK and in Scandinavian countries. Prevalence rates in the overall population have been reported to be >50% in France [14], 52.4% in Switzerland [15], and 59% in Germany [16], while rates in women of reproductive age have been reported to be 20.3% in Finland [17], 10.9% in Norway [9], 8.1% in England [18], 40% in Switzerland [15], and 54.3% in France [19]. Differences in the prevalence of *Toxoplasma* infection have been associated with warm and humid environments, cooking habits and the number of cats living outdoors, but the exact roles of these factors are still not fully understood [20,21]. In northern Greece, the climate is warm and humid during the summer, but is cold during winter, and raw or undercooked meat is eaten very rarely.

In 1966, Fulton et al. [22] found a high seropositivity rate of 60% in southern Greece with the Dye test, and a rate of 43% was found in northern Greece in 1972 with the same test [2]. A significant decline in the prevalence of *Toxoplasma* infection was observed in northern Greece in 1984 and 1994 (37.3% and 29.8%, respectively), dropping to 24.1% in 2004 in the present study. Various studies from Europe [23] and the USA [20] have also reported a decrease in the seroprevalence of *Toxoplasma* infection during the past 20–30 years. The reason for this is not clear, although it may
result from changes in the rate of exposure of susceptible individuals to tissue cysts or oocyst-contaminated environmental reservoirs. In northern Greece, this gradual decrease may be explained by the improved socio-economic situation. Reduced consumption of home-grown fruit and vegetables, wide consumption of pasteurised milk, and the increasing use of freezers in the home, may be some of the factors that have led to a decrease in human infection, as well as increased awareness and education with respect to eating adequately cooked meat, keeping cats, hygienic vegetable preparation and hand washing [24]. No recent studies in the general population have been reported from southern Greece.

In the present study, the prevalence rate in 2004 increased with age, from 3.2% in the group aged 1–4 years, to 56.4% in the group aged 60–69 years, with analogous increases in seropositivity in 1984 and 1994. There was a clear decline in the prevalence in each age group from decade to decade (Table 2). A cohort effect was observed for the group aged 20–29 years in 1984. The observed shift towards older age groups could be explained by the fact that increasingly large numbers of people in Greece live in apartments without gardens, so that children grow up away from gardens, soil and animals. However, other factors may also have influenced this shift, as different sources of infection are found in different age groups [25].

As with seroprevalence, the incidence of primary infection with *Toxoplasma* in the general population, as well as in women of reproductive age, seems to have decreased during the last 20 years in northern Greece. In the group of women of reproductive age, the incidence, as estimated by the cases of primary infection, decreased from 0.69%/pregnancy in 1984 to 0.55% in 2004, while the incidence estimated by the annual seroconversion rate was found to have declined from 0.81%/pregnancy in 1984 to 0.48% in 2004. Similar decreases have been reported from some other countries [23,26], with values ranging between 0.06% and 1.48% during pregnancy [19,20,27].

The prevalence of *T. gondii* infection showed a significant decreasing trend in northern Greece during the study period, resulting in a high (80%) proportion of non-immune women of reproductive age. Based on an estimated incidence in 2004 of 0.51%/pregnancy, an assumed maternofetal transmission rate of 23–50% [9,20,27], and an annual birth rate of 100 000 in Greece, it can be calculated that c. 408 pregnant women are infected annually with *Toxoplasma* during their pregnancy, and that 90–200 children are infected in utero by *T. gondii*. The present results are not representative of the entire country, so further studies should be performed to establish the complete picture of *Toxoplasma* infection in Greece. In parallel, it is also important to inform and educate the public on how to avoid possible risk-factors and prevent *Toxoplasma* infection.

REFERENCES


