



Serological evidence of *Toxoplasma gondii* infection among pregnant women in Auckland

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Abstract

Aim Severe congenital infection is a consequence of primary *Toxoplasma gondii* infection in early pregnancy. Antenatal screening is problematic because IgM antibody to *Toxoplasma* persists for months to years and thus may falsely indicate a recent infection. Serological screening for *T. gondii* infection is not currently included in routine antenatal testing in New Zealand. The aim of this study was to determine the prevalence of IgG and IgM antibody to *T. gondii* in pregnant Auckland women.

Methods Five hundred serum samples submitted for routine antenatal blood tests were tested anonymously for IgG and IgM antibodies to *T. gondii*. One hundred consecutive serum samples were tested from five age groups: <20, 21–25, 26–30, 31–35, >36 years. The number of positive IgM results that would have occurred if there were routine screening for toxoplasmosis was estimated for the year 2000 by multiplying the number of women giving birth in the respective age groups by the proportion with positive IgM results in these samples.

Results One hundred and sixty three (33%) women had IgG antibody to *T. gondii* and 12 (2.4%) also had IgM antibody. For the year 2000, if there had been routine screening for toxoplasmosis, 296 of 14 530 (2.03%, 95% CI: 1.8–2.2%) pregnant Auckland women would have had a positive IgM result.

Conclusions Screening would detect IgM antibodies in up to 2.2% of pregnant women. Significant and invasive further investigations would be required to identify the subset of pregnancies with fetal infection. Serological tests that are specific for recent primary infection are needed before routine screening could be considered for New Zealand. In the meantime, advice on how to avoid infection is necessary given that two thirds of pregnant Auckland women are susceptible to *T. gondii*.

Primary *Toxoplasma gondii* infection in pregnancy is usually asymptomatic, nevertheless infection may be transmitted to the fetus and cause severe damage.¹ Infections occurring in the first trimester have a 10% chance of transmission to the fetus and have the worst prognosis because of the risk of extensive central nervous system involvement.² Infection occurring in later pregnancy is transmitted to the fetus more frequently but does not cause such extensive damage. Some countries, such as France, Switzerland, Austria, Germany, Norway and Italy, screen pregnant women for *T. gondii* infection during pregnancy. Other countries including New Zealand do not screen.

Screening for toxoplasmosis is made difficult by the unusual persistence of the IgM antibody response.^{3,4} With most other infections, the predominate early response is IgM antibody, which then dwindles to undetectable levels within a few weeks. After primary *T. gondii* infection, however, IgM antibody frequently remains positive for many months or even years. A screening programme aimed at pregnant women in

their first trimester would ideally detect only very recent infection, since pre-conception infection is not a risk to the subsequent pregnancy. An antenatal screening programme would therefore need to include tests for IgM antibody. As a consequence of the persistent IgM response a number of women would be detected with *Toxoplasma* IgM in whom primary infection pre-dated conception. In these women the fetus is not at risk of congenital toxoplasmosis,^{5, 6} but the problem is how to distinguish them from women with true first-trimester infection.

In order to provide data on the immune status of pregnant women, 500 serum samples submitted for routine antenatal screening were tested for antibody to *T. gondii*.

Methods

Serum selection One hundred consecutive serum samples from each of five age groups (<20, 21–25, 26–30, 31–35, >36 years) were tested anonymously for antibodies to *T. gondii*. Those that were IgG positive were tested for IgM antibody. The samples had been submitted for the first routine set of antenatal blood tests.

Test methods Serum samples were tested in an enzyme immunoassay (EIA) on the AxSym system using the IMXII kit (Abbott Laboratories). Sera with positive IgM results were sent to the Immunology Department of Auckland Hospital for further testing. This comprised an in-house immunofluorescent assay (IFA) using reconstituted *T. gondii* trophozoites (BioMerieux) fixed onto pre-marked wells on clean slides. A 1:64 dilution of the serum sample, using rabbit anti-human IgG antiserum as the diluent, was added to the well. Slides were then incubated for 30 minutes at 37°C and rinsed. Specific, fluorescent labelled anti-IgM conjugate (Fluoline-M, BioMerieux) was then added and the slides incubated for further 30 minutes at 37°C. Subsequently, slides were washed, mounted and read using UV illumination. Fluorescence was recorded as negative, weakly positive, or positive. Sera were read without knowledge of the EIA result along with a random selection of negative sera. Sera IgM positive by both EIA and IFA methods were deemed IgM positive.

Estimation of screening results The last calendar year for which full information is available is 2000, when 14 530 mothers gave birth at National Women's and Middlemore Hospitals (personal communication, Maternity Services, 2003). To estimate the results of routine screening for toxoplasmosis the number of women giving birth in the respective age groups above was multiplied by the proportion with positive IgM results in our sample.

Statistics To assess the significance of trend the Cochran-Armitage Trend Test was used. Statistical analysis was performed using SAS release 8.0 Software (Cary, North Carolina).

The study was approved by the Ethics Committee North Health (reference number 97/118).

Results

One hundred and sixty three (33%) women had IgG antibody to *Toxoplasma* (Table 1). Sixteen samples that were IgM positive by EIA were sent for IFA testing and 12 (2.4%) were confirmed as being IgM positive. There was a trend for the prevalence of IgG antibodies to increase with age (Table 1, $p = 0.13$). In 2000, for the five age groups listed in Table 1, the estimated numbers of women with positive IgM giving birth were 49/1227, 141/2814, 0/4049, 84/4207, and 22/2233 respectively. We estimate, therefore, that 296 of 14 530 women (2.03%, 95% CI: 1.8–2.2%) would have had a positive IgM for *T. gondii* if routine screening had been performed. Thus, up to 2.2% of women could have initial antenatal serology consistent with recent infection.

Table 1. *Toxoplasma gondii* serology results of 500 pregnant Auckland women

Age group (years)*	IgG positive (%) [†]	IgM positive (%)
<20	36	4
21–25	24	5
26–30	29	0
31–35	34	2
>36	40	1
All ages	33	2.4

*100 serum samples tested in each age group; [†]p= 0.13 for trend of increasing seroprevalence with age

Discussion

Antenatal screening for infectious diseases presupposes there can be a better outcome for the baby. There are clear guidelines as to what constitutes a worthwhile screening programme,⁷ and understanding the disease incidence is an essential starting point. Other requirements are the availability of simple and safe tests that clearly differentiate between infected and non-infected individuals and, most importantly, the availability of effective interventions.

Antenatal screening in New Zealand currently includes rubella, hepatitis B and syphilis. In each of these infections there are acceptable interventions based on the known risk of adverse outcome to the fetus and the known efficacy of medical therapy. There are strong arguments for adding HIV testing to this programme because, with proper management, the risk of fetal infection can be drastically reduced. Unfortunately, the situation with toxoplasmosis is much less clear. First, the incidence of congenital toxoplasmosis in New Zealand is unknown, so the risks/benefits of a screening programme cannot be assessed. Second, there are no good data on the benefits of treatment during pregnancy.^{8–10} Third, current serological tests may not distinguish between recently acquired and remote infection.¹¹ Consequently, there is the very real possibility that more harm might result from positive screening tests than potential benefit from detection of pregnancies actually at risk.

Our study set out to answer two questions: what percentage of pregnant women in Auckland have antibody to *T.gondii* at the time of their first antenatal visit; and how many of these women have serological evidence of recent primary infection.

Testing for toxoplasmosis involves testing for IgG and IgM antibodies. With most infections, IgM antibody disappears some weeks after the primary infection so detection of IgM implies recent infection. With toxoplasmosis, the IgM antibody may persist for months or even years.¹² Attempts to increase the specificity for recent infection include testing for IgA antibody and testing for avidity of the IgG antibody.^{13–15} Avidity testing is based on the maturation of the antibody response over time, with low-avidity antibody being replaced by high-avidity antibody. The detection of high-avidity IgG antibody may be useful by allowing us to place infection before conception. This depends on the assay in question and the timing of the test.^{13,14} There are, however, only few data on the clinical outcome of children (n = 13) born to women who had high-avidity antibody in their first trimester.¹⁶ Although none of these children had evidence of *T. gondii* infection at follow up, all

the mothers had received spiramycin treatment during their pregnancy. It is unclear, therefore, whether these 13 children did not become infected because the maternal infection truly pre-dated conception, as suggested by the high-avidity IgG, or spiramycin protected the fetus, or whether the result was observed by chance since the probability of congenital infection following first-trimester infection is only about 10%. Clearly, more data are required on the reliability of IgG avidity testing.

Second-round tests involve looking directly for the *Toxoplasma* parasite, which in practice devolves to polymerase chain reaction (PCR) for *T. gondii* DNA.¹⁷ Searching for the parasite has its own problems, first and foremost being the rapidity with which *T. gondii* is cleared from the circulation in the immunocompetent host. A study looked for *Toxoplasma* DNA in the peripheral white blood cells of immunocompetent individuals with a known exposure date. *T. gondii* DNA could be recovered from approximately 33% of the patients over the 10 weeks following primary infection, with 53% of these positive results within five weeks of infection.¹⁸ Since most maternal infections are subclinical, the opportunities to test within that five-week period are rare. Even if acute maternal infection can be established unambiguously, this does not necessarily imply fetal infection.¹⁹ Attempts have been made to monitor fetal status by ultrasound imaging, but this has low sensitivity.²⁰ Currently, amniocentesis and testing for *T. gondii* by PCR is considered the most reliable diagnostic approach, with high positive and negative predictive values.^{21–26} Amniocentesis does, however, pose a risk to the pregnancy and requires considerable medical resources.

Our data show that 2% (range 1.8–2.2%) of women tested in the first trimester will have an antibody profile consistent with recent primary *T. gondii* infection. If one accepts that transmission to the fetus in the first trimester will occur in 10% of these infections, that implies some 2/1000 cases of first-trimester congenital toxoplasmosis in Auckland. Considering that first-trimester infection is the most severe and would be clinically apparent, this is clearly a gross overestimate, since this level of clinical disease is not occurring. Therefore, as a result of screening, up to 2.2% of pregnant women will have the anxiety of a potentially damaged baby and may undergo amniocentesis and/or termination of the pregnancy. The overwhelming majority of these procedures will be unnecessary.

Medical intervention is likewise of uncertain benefit.²⁷ Traditionally, spiramycin has been advocated for treatment in early pregnancy, being apparently risk free to the fetus and having putative parasitostatic properties.²⁸ It is hoped that the administration of this antibiotic will prevent *Toxoplasma* from replicating in the placenta and thus avert fetal infection. There have been no controlled trials of the effectiveness of spiramycin in preventing congenital toxoplasmosis; instead all efficacy data are based on comparison with historical untreated series. Treatment in later pregnancy generally includes either pyrimethamine/sulphadiazine plus supplemental folic acid or co-trimoxazole in a bid to achieve active treatment of the infected fetus and, particularly, to treat infection of the central nervous system.²⁹ Pyrimethamine/sulphadiazine treatment is not without side-effects and may be teratogenic as a consequence of severe bone marrow suppression unless adequate folic acid is supplied.

It is apparent that the risk of *T. gondii* infection currently does not fulfil the requirements that would justify its inclusion in an antenatal screening programme in New Zealand. Data on the incidence of congenital infection is sparse. We have

presented data on a small series of pregnant women in Auckland, but this is an urban community and figures from rural districts may be significantly different.³⁰ The current serological tests that might be offered in a screening programme lack the necessary discrimination between recent and remote infection. The consequences of this are the extensive and invasive investigations needed to assess the possibility of true first-trimester infection. Nor is there a proven medical intervention available, with scant data available on the efficacy of the currently recommended treatment protocols.

In view of these major barriers to screening, we suggest at this time that education for women on how to avoid *T. gondii* infection should be the preferred approach. Testing is best reserved for symptomatic patients, in whom the prior probability of infection favourably weights the positive predictive value of a positive IgM result.

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