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Duration of the IgM response in women acquiring *Toxoplasma gondii* during pregnancy: implications for clinical practice and cross-sectional incidence studies

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SUMMARY

We followed up a cohort of 446 toxoplasma-infected pregnant women to determine the median and variability of the duration of positive toxoplasma–IgM (immunoglobulin M) results measured by an immunofluorescence test (IFT) and an immunosorbent agglutination assay (ISAGA). IgM antibodies were detected for longer using the ISAGA test [median 12·8 months, interquartile range (IQR) 6·9–24·9] than the IFT (median 10·4, IQR 7·1–14·4), but the variability between individuals in the duration of IgM positivity was greatest for the ISAGA test. IgM-positive results persisted beyond 2 years in a substantial minority of women (27·1% ISAGA, 9·1% IFT). Variation in the duration of the IgM response measured by ISAGA and IFT limit their usefulness for predicting the timing of infection in pregnant women. However, measurement of IgM and IgG antibodies in a cross-sectional serosurvey offers an efficient method for estimating the incidence of toxoplasma infection.

INTRODUCTION

Toxoplasma gondii is a ubiquitous protozoan parasite acquired from undercooked meat, or from food or water contaminated by toxoplasma oocysts [1, 2]. Infection can be transmitted to the foetus by women who first acquire toxoplasmosis during pregnancy, although the risk of mother-to-child transmission depends on the gestational age at maternal infection [3]. As maternal infection is usually asymptomatic, serological tests for toxoplasma-specific immunoglobulin M (IgM) and IgG are used to identify women infected during pregnancy. IgM antibodies can be detected approximately 14 days after acquisition of toxoplasma infection [4] and decline to undetectable levels several months later depending on the test used [1, 4]. In contrast, IgG antibodies are detectable 14 days after the first positive IgM test [3] and persist indefinitely. Consequently, IgM results are widely used, together with other serological results such as IgG titre and avidity [1, 4] to estimate the timing of maternal infection.

Information on the timing of infection provided by IgM results can also be used to estimate the incidence of infection in cross-sectional studies based on a single serosurvey [5]. Alternative methods for measuring the incidence of maternal infection are problematic. Follow-up studies to measure seroconversion (change from negative to positive antibodies) are expensive and time-consuming [6]. On the other hand, modelling using cross-sectional data on age-specific IgG prevalence overestimates incidence due the sharp decline in seroprevalence in pregnant women over the last 3 decades [7, 8].

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Use of IgM results for these clinical and epidemiological applications requires information on the average duration of the IgM response and how much this varies between individuals. For example, a test result with a long mean duration but with little variability might be more useful for timing maternal infection than one with a short mean duration but with large variability.

Several studies have reported a minimum duration of specific IgM, but none have followed up subjects for long enough to detect reversion to negativity in more than a handful of individuals [9–11]. No previous reports of the variability of toxoplasma-specific IgM duration could be found. We therefore studied a large cohort of women who acquired toxoplasma infection during pregnancy and whose IgM status was followed up long-term. The aim was to determine the median and variability of the duration of positive toxoplasma–IgM results measured by two tests: an automated immunofluorescence test (IFT) [12] and an immunosorbent agglutination assay (ISAGA–IgM; bioMérieux, Marcy, France) [13]. We show how the results can be used to help clinicians identify women at high risk of post-conceptional toxoplasma infection and to estimate the incidence of maternal infection in cross-sectional serosurveys.

MATERIALS AND METHODS

Study population

The study is based on a cohort of 788 toxoplasma-infected pregnant women identified by routine prenatal testing for maternal toxoplasma infection in Lyon, France between 1987 and 1995. Over 90% of the women received antibiotic treatment for toxoplasmosis during pregnancy. Serum samples were taken from the mother at monthly follow-up visits during pregnancy and, for a subsample, when the child was seen for clinical assessment (usually at the 2-month paediatric assessment) in order to compare maternal antibody responses with those of the child. Further details of the cohort, and details of the ISAGA and IFT are reported elsewhere [3, 12–14].

Measurement of IgM duration

For each test (ISAGA and IFT), four dates were extracted: the last IgM-negative test and first IgM-positive test (these dates bound the interval in which initial maternal seroconversion occurred), and the last IgM-positive test and first IgM-negative test (these dates bound the interval in which the change to IgM negativity occurred). Women not observed to seroconvert (change from negative to positive IgM) were deleted from the analysis unless: (*a*) their child was infected, in which case the last negative test was assumed to have occurred at conception; and (*b*) the first IgM test was positive but all IgG tests on the same date were negative, in which case the last negative IgM test was assumed to be dated 3 weeks previously. Not all women were followed up long enough to observe the change back from positive to negative IgM antibody (right-censored cases) but provide information on the minimum duration of IgM positivity and were included in the analysis (see Statistical analysis section below). In cases where only one positive sample was observed, the date of the first and last IgM-positive test was the same. We used the manufacturer's recommended cut-off for the ISAGA test and previously reported cut-offs for the IFT [12]. The cut-off values used for the ISAGA were 0–5 IU/ml (negative), 6–8 IU/ml (equivocal) and 9–12 IU/ml (positive). For the IFT this was 0 IU/ml (negative), 0–6·4 IU/ml (equivocal) and more than 6·4 IU/ml (positive). Equivocal values were excluded from the analysis since treating them arbitrarily as positive or negative test results would lead to spuriously precise observations and might bias the point estimates of the cumulative distribution function of duration of IgM positivity as well as reducing their standard errors.

Statistical analysis

Standard survival analysis could not be used to determine the duration of IgM-positive results as the exact moment of change of IgM antibody from negative to positive or back from positive to negative was not observed. Analyses were therefore performed using non-parametric methods for doubly interval-censored data [15].

We determined the duration of IgM positivity for each test type, whether the woman gave birth to an infected child or not, and whether she had received prenatal treatment with a combination of pyrimethamine–sulphonamide or spiramycin. These latter analyses were based on the hypothesis that women with an infected foetus experience a more pronounced or prolonged immunological stimulation and, secondly, based on clinical observations that pyrimethamine suppresses the immunological response.

In order to test for differences in the duration of IgM positivity between tests, the child's infection

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status and type of treatment, we used the multiple imputation approach to Cox regression with doubly censored data proposed by Pan [16]. We used 1000 imputed data-sets in all the analyses and performed the goodness-of-fit tests for the imputed Cox proportional hazard models based on a $\chi^2_{(1)}$ statistic. The latter tests for departures of the proportionality of hazard assumption required by the Cox models. The Cox regression models and the goodness-of-fit tests were calculated using *R* in a Unix environment; the programs were modified versions of those reported by Pan [16].

To estimate incidence from one cross-sectional sample we used the formula for estimating recent incidence from Kaplan and Brookmeyer [5]. However, for this method to be useful the average duration of IgM positivity needs to be sufficiently small (e.g. maximum 2 years). As some women in this study did not revert to negativity, the average duration of positive IgM results presented in this study is infinite. We therefore modified the method of Kaplan and Brookmeyer to adjust for positive IgM results for longer than 2 years.

To estimate the incidence in the period t times unit prior to the sampling date the following modification to Kaplan and Brookmeyer's estimate can be used:

$$r(-\tau_t)=\frac{p\pi(1-\mu_t)}{(1-p)\tau_t}, \qquad (1)$$

where $r(-\tau_t)$ denotes the incidence as a fraction of the uninfected population between the sampling date and the period t time units prior to the sampling date, p is the fraction of IgG-positive persons in the population (prevalence of the infection), π is the fraction of IgM-positive persons among IgG-positive persons, τ_t is the average duration of IgM positivity, given that the duration was at most t time units, and μ_t is the fraction of IgM-positive persons with an IgM-positive duration longer than t time units, among all IgM-positive persons. μ_t and τ_t can be computed from the results in this paper whilst p and π can be estimated from one cross-sectional sample. The modification adjusts for IgM positivity longer than t time units by multiplying the fraction of IgM-positive persons among IgG-positive persons in the serosurvey with the estimated fraction of IgM-positive persons with IgM positivity shorter than t time units. The average duration of IgM positivity (τ_t) is then calculated, conditional on the duration lasting at most t time units. The method assumes that the infection rate is approximately constant over the sampling period.

Let n, n^+, and n_M be the numbers of individuals tested, IgG positive and IgM positive among those IgG positive respectively. Then the estimated prevalence is $\hat{p}=n^+/n$, and $\hat{\pi}=n_M/n^+$ is the estimated fraction of IgM positives among the IgG-positive individuals. $\hat{\mu}_t$ is the estimated fraction of IgM-positive persons with an IgM positivity longer than t time units among all IgM positives and $\hat{\tau}_t$ is the estimated average duration of IgM positivity conditional on it being less than or equal to t time units (both are estimated in this paper). Applying the delta method [17] to the logarithm of eqn (1), assuming that $\hat{\mu}_t$ and $\hat{\pi}$ are independent, yields an estimate for the asymptotic variance of the estimate of the log-incidence as:

$$\hat{v}=\widehat{\mathrm{var}(\ln(\hat{r}(-\hat{\tau}_t)))}=\frac{1}{(1-\hat{p})\hat{p}n}+\frac{1}{n_M}\left(\frac{\hat{\mu}_t}{1-\hat{\mu}}+\frac{1-\hat{\pi}}{\hat{\pi}}\right)+\frac{\widehat{\mathrm{var}(\tau_t)}}{\hat{\tau}_t^2},$$

and an asymptotic 95% confidence interval (CI) is given by $[\hat{r}(-\hat{\tau}_t)e^{-1\cdot96\sqrt{\hat{v}}},\ \hat{r}(-\hat{\tau}_t)e^{1\cdot96\sqrt{\hat{v}}}]$.

RESULTS

Of the 788 toxoplasma-infected women in the cohort, 342 were excluded as they were not observed to change from negative to positive IgM (no initial negative test was available) and did not give birth to an infected child. Of the remaining 446 women who seroconverted during or shortly before pregnancy, 182 (41%) gave birth to a child with congenital toxoplasmosis [241 were uninfected and 23/446 (5%) children had insufficient follow up to determine congenital infection status]. Prenatal treatment was prescribed to 415/446 (93%) women, of whom 98/415 (24%) were prescribed pyrimethamine–sulphadiazine. The median duration of follow-up from the first negative IgM test or conception to the last IgM test was 11 months (range 1–81 months).

Table 1 shows the number of women analysed according to test type. As testing depended on laboratory practice, women were not always tested with both tests. A total of 102 women (25%) reverted to a negative test result with the ISAGA test and 105 (29%) did so with the IFT. Figure 1 shows the cumulative distribution function of the duration of IgM positivity according to the two tests. The difference in duration between both tests was significant ($P<0{\cdot}0001$). The y-axis shows the percentage of women with IgM positivity lasting less than the

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Table 1. *Number of women analysed according to IgM test type* (*446 women were studied*)

Pattern of results*	ISAGA	IFT
−+− or −++−	102 (25%)	105 (29%)
−+ or −++ (censored)	312 (75%)	254 (71%)
Total	414	359

ISAGA, Immunosorbent agglutination assay; IFT, Immunofluorescence test.
* −, Negative; +, positive.
−+− or −++−, Women in whom the initial change from negative to positive IgM was observed, as well as the change back from positivity to negativity.
−+ or −++, Women in whom the initial change from negative to positive IgM was observed, but were not followed up long enough to observe any change back from positivity to negativity.
−+− and −+, Only one positive test observed.
−++− and −++, More than one positive test observed.

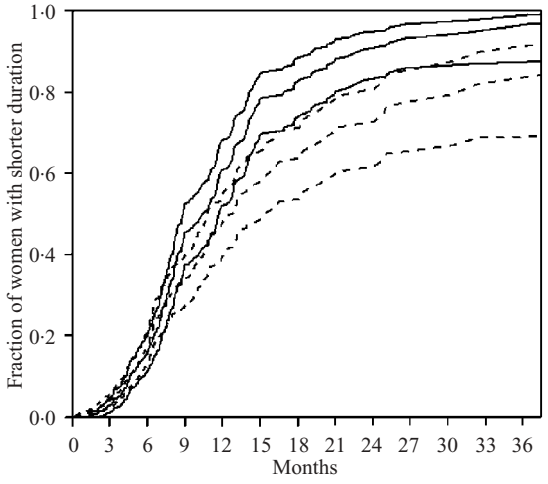

Fig. 1. Duration of detection of IgM antibodies and 95% CIs using the IFT (—), and the ISAGA test (– – –).

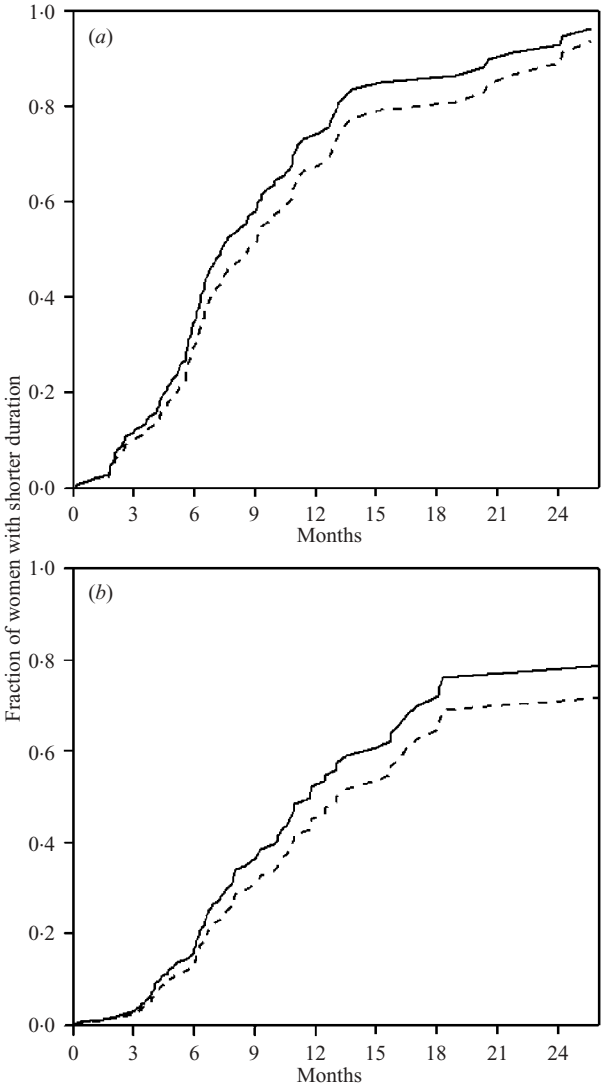

Fig. 2. Comparison of the duration of detection of IgM antibodies in women who gave birth to infected (– – –) *vs.* uninfected children (—). (*a*) Compares women tested with the IFT; (*b*) compares women tested with the ISAGA test.

time-period plotted on the *x*-axis. IgM antibodies were detected for longer by the ISAGA test compared to the IFT. For example, 50% of women had positive ISAGA results for less than 12·8 months [interquartile range (IQR) 6·9–24·9 months] and positive IFT results for less than 10·4 months (IQR 7·1–14·4 months). The steeper the gradient of the slope of the cumulative distribution function, the less variability between individuals in the duration of IgM. Comparison of the gradient for the IFT and ISAGA test shows that the variability between individuals was smaller for the IFT than the ISAGA test. An estimated 27·1% of women (95% CI 19·2–38·3) had a positive ISAGA result, and 9·1% (95% CI 4·9–16·5) had a positive IFT result lasting longer than 2 years. Confidence intervals around these estimates are wide due to the small number of women with a sufficiently long follow-up.

Figure 2(*a*, *b*) shows that IgM antibodies were detected for longer using both tests in women who gave birth to an infected, compared to an uninfected, child. However, differences in the duration of positivity according to congenital infection status were not significant for either test ($P=0{\cdot}34$ and $P=0{\cdot}32$ for IFT and ISAGA respectively). Using the ISAGA test (Fig. 2*b*), IgM was detected for less than 11·7 months (IQR 6·7–18·5 months) in 50% of women with an uninfected child compared to 13·1 months

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Table 2. *Estimates of the regression coefficient and the goodness-of-fit tests*

Comparison	Regression coefficient $\hat{\beta}$	s.e. $\hat{\beta}$	*P* value	Goodness-of-fit test $\chi^2_{(1)}$	*P* value
IFT *vs.* ISAGA	−0·626	0·140	<0·0001	0·390	0·53
Congenital infection status					
IFT	−0·192	0·202	0·34	0·005	0·94
ISAGA	−0·206	0·212	0·32	0·001	0·99
Treatment (spiramycin alone *vs.* pyrimethamine–sulphonamide)					
IFT	−0·069	0·2362	0·76	1·261	0·26
ISAGA	−0·278	0·2625	0·29	0·027	0·86

ISAGA, Immunosorbent agglutination assay; IFT, Immunofluorescence test.

(IQR 7·5–31·3 months) for 50% of those with an infected child. Corresponding values for the IFT (Fig. 2*a*) were 7·3 months for 50% of women with an infected child (IQR 5·7–13·3) compared to 8·6 months for 50% of those with an uninfected child (IQR 5·2–12·5 months).

The distribution function for IgM duration in women treated with pyrimethamine–sulphonamide compared to spiramycin was similar for both test types [Fig. 3(*a*, *b*); $P=0{\cdot}76$, and $P=0{\cdot}29$ for IFT and ISAGA respectively].

Table 2 shows the values for the regression coefficients for the three comparisons between tests, congenital infection status of the child, and type of prenatal treatment, as well as the goodness-of-fit statistic. The *P* value of the goodness-of-fit statistic for all comparisons performed was bigger than 0·05 indicating that there was no evidence against the proportional hazards assumption.

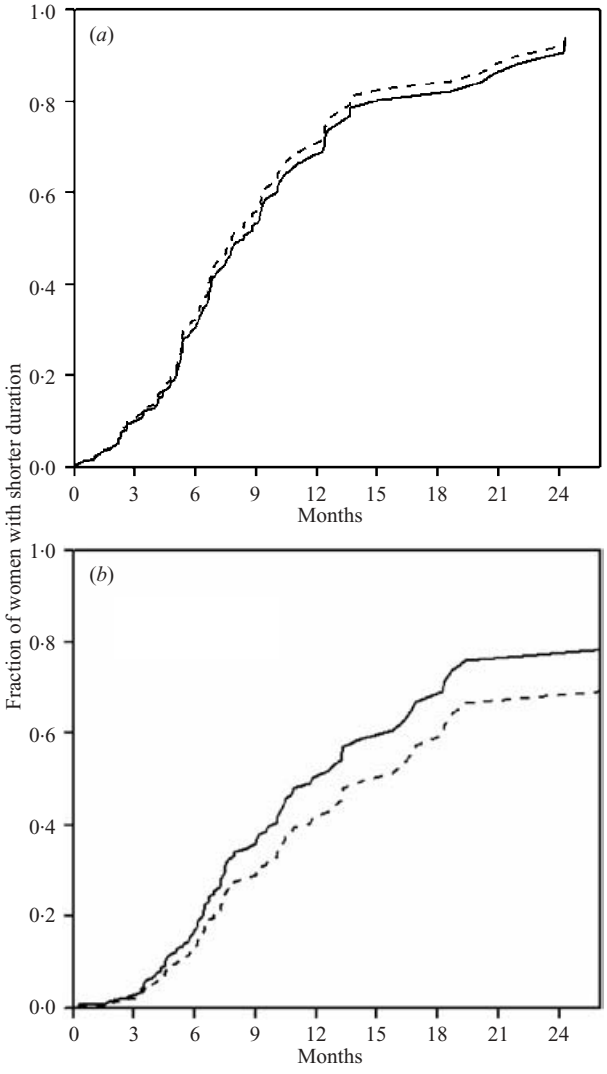

Fig. 3. Comparison of the duration of detection of IgM antibodies in women treated with pyrimethamine–sulphon amide at any point during pregnancy (—) *vs.* those treated with spiramycin alone (– – –). (*a*) Compares women tested with the IFT; (*b*) compares women tested with the ISAGA test.

DISCUSSION

IgM antibodies were detected for a significantly longer time using the ISAGA test than the IFT, but the variability between individuals in the duration of IgM positivity was greatest for the ISAGA test. There was a substantial minority of women with long-term persistence of IgM of unknown duration. There was no significant difference in the duration of IgM between women who gave birth to an infected, compared to an uninfected child, nor between women treated with pyrimethamine–sulphonamide or spiramycin, although small sample size limited the power to detect a difference.

The implications of our findings for a woman with a positive IgM and IgG test at her first prenatal visit are that the risk of post-conceptional infection is small but uncertain. Using the estimates in Figure 1, the risk of post-conceptional infection for a woman who is first tested at 3 months' gestation, is less than 1·3% (95% CI 0·6–2·1) given a positive ISAGA IgM result, and less than 3·1% (95% CI 0·7–4·6) given a positive IFT result. A positive IgM test result based on a single sample in the first trimester therefore provides insufficient information to identify women

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in whom further investigation, termination of pregnancy or treatment for toxoplasma infection may be justified.

A strength of our study was that measurement of IgM was routinely performed and was not conditional on clinical characteristics in the mother or child. In addition, the interval during which seroconversion occurred was clearly defined. A weakness is that, as 93% of women were prescribed treatment during pregnancy, we were unable to determine IgM duration in untreated women. Previous statements that treatment suppresses the IgM response [4, 18] appear to be based on clinical experience and no evidence has been published of differential effects according to the type of treatment.

This is the first published report of which we are aware of the distribution of the duration of IgM positivity measured by ISAGA or IFT in a population followed for long enough to detect reversion to negativity in more than five individuals. Nevertheless, comparison of previous follow-up studies of individuals with well-documented dates of infection can be instructive. Jenum et al. [9] found that 25% (5/27) of women reverted to ISAGA negative results between 14 and 31 weeks after infection, in agreement with our estimate (see Fig. 1) of 25% with IgM lasting less than 7 months (30 weeks). In contrast, Sulzer et al. [10] reported that 3/16 (19%) individuals reverted to IgM negativity by the IFT within 10·5 months of a waterborne outbreak of toxoplasmosis, whereas we estimated that 50% of women were negative 10·4 months after infection (Fig. 1). The discrepancy may reflect error due to the small number studied by Sulzer et al. Alternative explanations include prolonged persistence of IgM due to infection acquired by ingesting oocysts compared to tissue cysts from undercooked meat, which is most commonly associated with infections in pregnancy [2]. Further possibilities are improved sensitivity of the test or gender differences in immune response [19–21], as Sulzer et al.'s report was confined to men.

Our findings can be used to estimate the incidence of toxoplasma infection in cross-sectional studies based on a single serum sample from individuals tested for specific IgG and IgM antibodies, using statistical methods reported elsewhere [5, 22, 23]. Because of the risk of false-positive IgM results [1] positive tests should be confirmed by a second test and only included if the individual is also IgG positive. In view of the marked geographical and secular trends in seroprevalence, this method offers a low-cost, standardized approach with which to compare the burden of disease in different populations and to evaluate preventive measures. However, extrapolation of the method beyond pregnant women in a western industrialized country to other populations should be made with caution in view of the hypothesis that differences in the immune response or type of organism exposure may affect the duration of IgM seropositivity. Investigation of the latter would be assisted by tests able to distinguish between infection acquired from oocysts or tissue cysts.

The sample size needed for such a study depends on the prevalence of toxoplasmosis, the fraction of IgM-positive results among IgG-positive individuals,

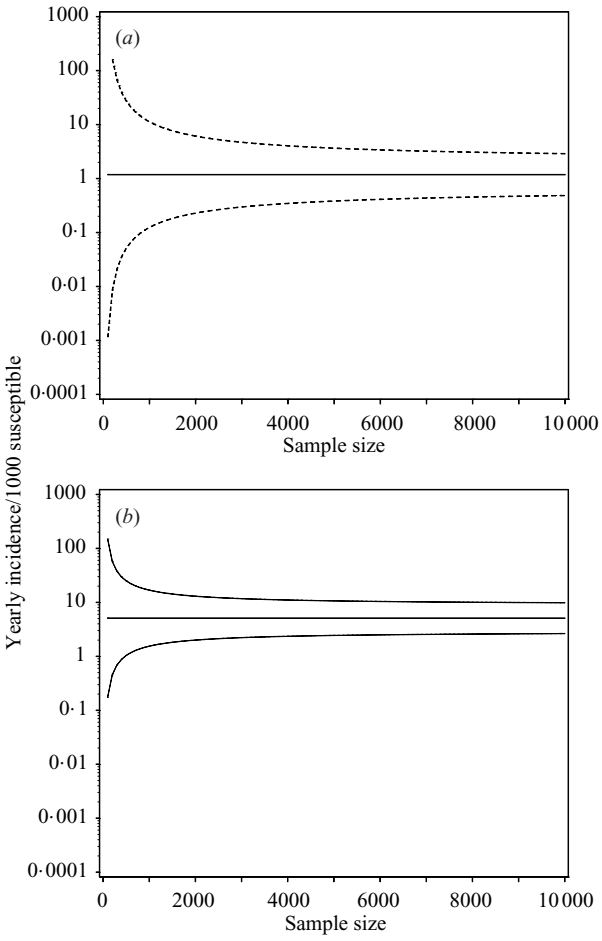

Fig. 4. The 95% CIs are given for the estimated yearly incidence (*y*-axis) from one cross-sectional sample using the IgM IFT and assuming a fraction of IgM positives among IgG positives of 0·8% (*a*) and 3·4% (*b*). Further assumptions are: a prevalence of IgG positives of 10% in the population, average duration of IgM positivity, given that the duration is shorter than 2 years equals 8·3/12 years (variance 5·8/144) and the proportion of women whose IgM-positive results lasts longer than 2 years is 9·1%.

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and the incidence and average duration of IgM positivity. For these calculations, average duration of ISAGA positivity, given that the duration was shorter than 2 years, was 10·2 months (95% CI 10·0–10·4) and for IFT 8·3 months (95% CI 7·2–8·9). The estimated proportion of women with a duration longer than 2 years was 27·1% (95% CI 19·2–38·3) with the ISAGA test, and 9·1% (95% CI 4·9–16·5) with the IFT. Figure 4(*a*, *b*) shows the 95% CIs around the estimated incidence using the above-mentioned figures for the IFT according to sample size. To illustrate the method, we assumed that the prevalence of IgG was 10%. We used two sources that give different results for the proportion of IgG-positive individuals who were IgM positive (3·49% [24], Fig. 4*b* and 0·81% [25], Fig. 4*a*). The figures show that for a sample size of 10 000, given the assumptions above, the estimated incidence would be 1·1/1000 susceptible women/year (95% CI 0·5/1000 to 2·8/1000) and for Fig. 4*b*, 5·1/1000 susceptible women/year (95% CI 2·6/1000 to 9·8/1000). The sample size needed could be further reduced if additional research provided more precise estimates for the mean duration of IgM positivity.

CONCLUSION

Variability in the duration of the IgM response measured by ISAGA and IFT limit their usefulness for predicting the timing of infection in pregnant women. The decision to terminate the pregnancy or offer therapy to reduce the risk of transmission should not be based on a single IgM- and IgG-positive test result alone. However, measurement of IgM and IgG antibodies in a cross-sectional serosurvey offers an efficient method for estimating the incidence of toxoplasma infection in populations.

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