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Seroepidemiology of *Toxoplasma* infection in a metropolitan region of Brazil

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SUMMARY

Seroprevalence data from a representative population were used to estimate the annual incidence of congenital toxoplasmosis in São Paulo Metropolitan Region (SPMR). Retrospective anti-toxoplasma IgG serological analysis was conducted to determine age-dependent seroprevalence, force of infection, average age of acquisition of infection and curve of decay of maternally derived antibodies. Seroprevalence was used to calculate the number of new infections. Toxoplasmosis in pregnant women was estimated by total number of deliveries in a given year as a proxy for the number of pregnancies per year. *Toxoplasma* seroprevalence was 64·9% in women of childbearing age. Average age of acquisition of toxoplasmosis was 10·74 years. The estimated annual incidence of congenital toxoplasmosis varied from 9·5 to 10·6/1000 births in the studied period. The toxoplasmosis seroprevalence model allowed a good incidence estimation of congenital disease in SPMR compared to other published data, indicating that this mathematical approach is useful in calculating the potential demand of congenital disease due to *Toxoplasma gondii* in a given community.

Key words: Epidemiological parameters, incidence, mathematical modelling, seroepidemiology, toxoplasmosis.

INTRODUCTION

Toxoplasmosis is an infection that occurs worldwide, with higher incidence in tropical areas. On the other hand, incidence decreases in cold areas and high latitude. It is a common infection in Central and South America, especially in regions with a moderate climate [1].

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The infection is caused by *Toxoplasma gondii*, an obligate intracellular protozoan and may take several different forms: tachyzoites which rapidly multiply and destroy tissue during acute infection; bradyzoites which slowly multiply in body tissues, leading to dormant forms – cysts, and oocysts, which are excreted by recently infected cats in their faeces. It is one of the most evident members of the phylum with about a third of the human population being chronically infected [2, 3]. The parasite can be transmitted to the foetus by the passage of tachyzoites through the placenta [1].

The age-specific prevalence has been decreasing in some European countries over the past three to four decades [1], but Ades & Nokes pointed out that in

England stability has been observed since the 1970s after a sixfold decay in prevalence prior to that time [4]. This is a demonstration that toxoplasmosis varies from country to country, and even from time to time in the same country.

Many studies have shown that 50–80% of Brazilian women that are pregnant or of childbearing age have antibodies against *T. gondii* [5]. Brazil is a very large country, with heterogeneous access to healthcare and with considerable demographical, socioeconomic and cultural differences, which may explain variations in diverse studies and localities.

A number of factors influence the level of exposure to *T. gondii* in the population, e.g. ingestion of raw or poorly cooked meat, intake of contaminated vegetables, close association with cats [6], ingestion of non-filtered water, rural residential location [7] and contact with contaminated ground [8].

Toxoplasmosis has a significant impact from the public health perspective, mainly due to the risk of disease transmission through pregnancy [9]. Congenital infection still occurs, despite the availability of diagnostic examinations and therapy worldwide. In most cases it is the result of primary infection of a pregnant woman [2].

Vertical transmission of *T. gondii* may cause important morbidity and mortality to the foetus and the newborn, but in most cases the infants are asymptomatic at birth, developing the disease at any age [3]. The main frequent manifestations of congenital disease are learning and visual disabilities [10].

Worldwide, 3–8/1000 live births are infected in the uterus. There are many risk factors for the infection of the foetus, including the rate of transmission, mother's age, climate, area of residency, lifestyle, strain virulence, education and eating habits. Thus, this combination leads to marked differences even within countries [9, 11].

In Guatemala the estimated incidence of congenital toxoplasmosis reported in a study carried out in newborns was 10·9/1000 live births, although the authors believe that the incidence found was lower than the expected because the region is known for a high incidence of this infection among pregnant women [12].

Regardless of the high seroprevalence found in women of childbearing age in Brazil, few data are available on the actual incidence of congenital toxoplasmosis. Existing studies show an incidence of 0·33–1·96/1000 live births, based on the detection of anti-toxoplasma IgM antibodies from newborn blood samples [13].

The aim of this study is to estimate the annual incidence of congenital toxoplasmosis occurring in São Paulo Metropolitan Region (SPMR), utilizing mathematical modelling, from 1984 to 2006, using seroprevalence data from a representative population.

MATERIAL AND METHODS

The community sampled

The community chosen for the seroprevalence study was the population of Caieiras, a city in SPMR. Caieiras has a population profile representative of the SPMR population, i.e. >90% live in the urban area, social structure is very heterogeneous and the economy is based on industries, trade and services.

Individuals stratified by age were randomly sampled within dwellings from administrative regions in a two-level cluster design. Out of the 62 administrative regions in the city, with 150 dwellings each, 32 were randomly selected. Further details on this population sampling have been published elsewhere [14].

For the purpose of this study, the community sampled was divided in: infants aged 0–15 months (newborns in the first hour of life; 0·5 month; 1, 2, 3, …, 15 months); and individuals aged 1–39 years (1, 2, 3, 4, 5–9, 10–14, 15–19, 20–24, 25–29, 30–39 years). It was assumed 10 years of age onwards as possible for childbearing [15–17].

Blood was collected by a vacuum-containing system or by 'butterfly needle' venepuncture for children aged <2 years. Sera obtained after centrifugation of clotted samples were frozen and stored at −20 °C for up to 5 years.

Serological data

To determine the presence of antibodies against *T. gondii* in each person, latex indirect agglutination (Toxoreagent; Eiken Co. Ltd, Japan) was used. This is a method for specific antibodies to this parasite in which latex particles are coated with toxoplasma antigen to form agglutinating patterns in the presence of specific antibody. The result was considered positive for antibody titres >1:32, according to the manufacturer's instructions. This serological method has a sensitivity and specificity of 89·6% and 91·4%, respectively [18].

SPMR statistics

Located in the Southeast Region of São Paulo state, Brazil, SPMR comprises approximately 10% of the

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Brazilian population, with 39 municipalities in an area of 8051 km². SPMR is the largest urban conglomerate in South America, ranking among the largest megalopolises in the world. More details can be found in Fundação Seade database [19]. The SPMR population ranged from about 13 124 000 inhabitants in 1984 to almost 19 356 000 in 2006 [20].

From the Brazilian Statistics Institute (IBGE) database (1984–2006), the total number of deliveries $D(y)$ (newborns and stillborns) of each year y was taken as the number of pregnant women per year. Deliveries are reported in relation to the parturient age group, i.e. <15, 15–19, 20–24, 25–29, 30–34 and 35–39 years. Details can be found in the IBGE database [21–23].

Data management

Serological data were used to estimate the proportion of seropositive infants (aged 0–15 months) and the proportion of seropositive individuals (aged 1–39 years). The respective 95% confidence intervals (CI) were calculated.

An exponential function was chosen to represent the seroprevalence profile for infants, fitted to the following equation [24]

$$M(a) = M_0 + M_1 e^{-ka}, \tag{1}$$

where $M(a)$ is the proportion of seropositive children at age a, M_1 is the amplitude of the curve, k is the rate of change of the proportion, and M_0 is a fitting parameter.

To represent the seroprevalence profile of individuals aged 1–39 years, $S^+(a)$, serological data were fitted to the following the equation [25]

$$S^+(a) = 1 - \exp\left\{\frac{k_1}{k_2^2}\left[(k_2 a + 1)e^{-k_2 a} - 1\right]\right\}, \tag{2}$$

where k_1 and k_2 are the parameters to be fitted [24]. The age-dependent force of infection $\lambda(a)$ is defined as the *per capita* rate in which susceptible individuals acquire infection per unit of time. In this study $\lambda(a)$ was obtained from $S^+(a)$, from a catalytic model [26], according to

$$\lambda(a) = \frac{dS^+(a)}{da}[1 - S^+(a)]^{-1}, \tag{3}$$

Assuming that the force of infection reaches a peak at an age which corresponds to a maximum number of infections, with a subsequent decline in higher ages, we can take the model [27] modified from Farrington [25]

$$\lambda(a) = k_1 a e^{-k_2 a}, \tag{4}$$

The average age at infection can be estimated by [24]

$$A = \frac{\int_0^L a\lambda(a)[1 - S^+(a)]da}{\int_0^L \lambda(a)[1 - S^+(a)]da}, \tag{5}$$

in which L was taken as 39 years, the maximum age observed from the serological data.

The increase in the proportion of seropositivity between ages a_m and a_n was used to calculate $\Psi(a_m, a_n)$ by [28, 29]

$$\Psi(a_m, a_n) = S^+(a_n) - S^+(a_m), \tag{6}$$

IBGE computes the number of births from women in age intervals a_m to a_n (10–14, 15–19, 20–24, 25–29, 30–34, 35–39 years).

The number of pregnant women in a given age group per year y, $G(a_m, a_n; y)$, was then multiplied by $\Psi(a_m, a_n)$, to estimate the number of new cases of toxoplasmosis in pregnant women, $T(a_m, a_n; y)$, so

$$T(a_m, a_n; y) = G(a_m, a_n; y)\Psi(a_m, a_n), \tag{7}$$

The average probability of vertical transmission of toxoplasmosis is 0·29 (95% CI 0·25–0·33), for any gestational period [30], assumed as the probability of a congenital infection f. Then we may estimate the number of new congenital toxoplasmosis infections as

$$C(a_m, a_n; y) = fT(a_m, a_n; y), \tag{8}$$

Finally we calculate the annual number of congenital toxoplasmosis cases per 1000 births $E(y)$ by

$$E(y) = \frac{\sum_{a_m, a_n} C(a_m, a_n; y)}{D(y)} \times 1000. \tag{9}$$

This protocol was approved by the Ethics Committee (CAPPesq) of Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo (process number 1090/07).

RESULTS

Table 1 presents the results obtained from the analysis of 199 infants and shows the proportion of seropositive infants $M(a)$. Figure 1 shows the continuous function that represents the decrease in the proportion of seropositive infants $M(a)$, as an effect of

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Table 1. *Proportion seropositive for toxoplasmosis from 199 infants aged 0–15 months, Caieiras, São Paulo state, Brazil, 1990*

Age (months)	Sample size	Sero-positive	Proportion seropositive
0	39	26	0·67
0·5	6	4	0·67
1	7	3	0·43
2	10	4	0·40
3	9	3	0·33
4	9	1	0·11
5	8	0	0·00
6	12	1	0·08
7	21	1	0·05
8	18	0	0·00
9	13	0	0·00
10	18	1	0·06
11	14	0	0·00
12	3	0	0·00
13	2	0	0·00
14	3	0	0·00
15	7	0	0·00
Total	199	44	—

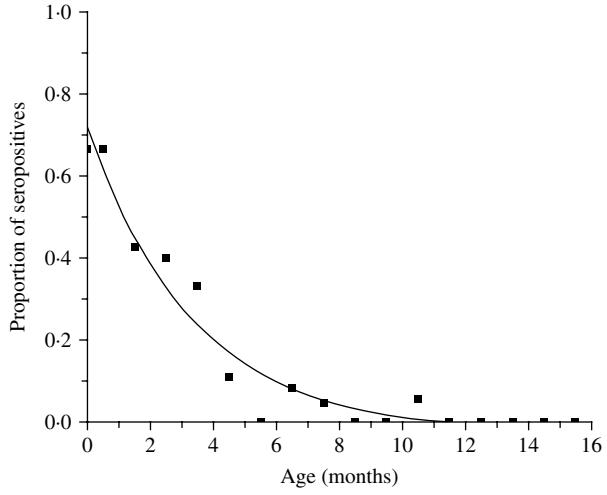

Fig. 1. Proportion seropositive $M(a)$, and decay of maternal antibodies in children from 0 to 15 months, Caieiras, 1990. Fitting parameters: $M_0 = -0{\cdot}025$ (S.E. $= 0{\cdot}027$), $M_1 = 0{\cdot}746$ (S.E. $= 0{\cdot}044$) and $k = 0{\cdot}299$ month^{-1} (S.E. $= 0{\cdot}046$ month^{-1}).

the decay of maternally derived antibodies. The fitting parameters for the infants' seroprevalence curve are $M_0 = -0{\cdot}025$ (S.E. $= 0{\cdot}027$), $M_1 = 0{\cdot}746$ (S.E. $= 0{\cdot}044$) and $k = 0{\cdot}299$ month^{-1} (S.E. $= 0{\cdot}046$ month^{-1}) ($\chi^2 = 0{\cdot}042$, D.F. $= 14$, $P = 1{\cdot}0$).

The overall positivity of toxoplasmosis in 250 sera samples from individuals aged 1–39 years was 26·8% (67/250). Table 2 shows the seroprevalence distributed by age group. The fitting parameters of the seroprevalence curve obtained were $k_1 = 0{\cdot}0237$ yr^{-2} (S.E. $= 0{\cdot}0031$ yr^{-2}) and $k_2 = 0{\cdot}140$ yr^{-1} (S.E. $= 0{\cdot}024$ yr^{-1}) ($\chi^2 = 5{\cdot}6$, D.F. $= 8$, $P = 0{\cdot}7$).

Table 2. *Age-specific seroprevalence of toxoplasmosis in 250 individuals aged 1–39 years, Caieiras, São Paulo state, Brazil, 1990.*

Age (years)	Sample size	Sero-positive	Sero-prevalence	95% CI
1	42	0	0·000	0·00–0·08
2	41	2	0·049	0·01–0·17
3	33	2	0·061	0·01–0·20
4	24	5	0·208	0·07–0·42
5–9	36	10	0·278	0·14–0·45
10–14	4	2	0·500	0·07–0·93
15–19	13	10	0·769	0·46–0·95
20–24	24	16	0·667	0·45–0·84
25–29	13	8	0·615	0·32–0·86
30–39	20	12	0·600	0·36–0·81
Total	250	67		—

CI, Confidence interval.

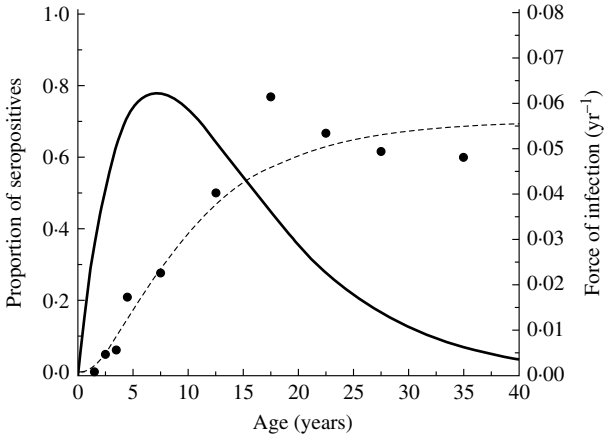

Fig. 2. Toxoplasmosis seroprevalence curve for Caieiras city, 1990. Fitting parameters: $k_1 = 0{\cdot}0237$ yr^{-2} (S.E. $= 0{\cdot}0031$ yr^{-2}) and $k_2 = 0{\cdot}140$ yr^{-1} (S.E. $= 0{\cdot}024$ yr^{-1}), and the force of infection obtained from equation (3) for the community under study.

Figure 2 shows the seroprevalence curve and the force of infection obtained from equation (3) of the community under study. The average age of acquisition of toxoplasmosis in this community, calculated using equation (4) was 10·74 yr (S.E. $= 0{\cdot}71$ yr).

Table 3 shows the increase in seroprevalence due to new infections $\Psi(a_m, a_n)$ between ages a_m and a_n obtained from the seroprevalence curve. Figure 3

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Table 3. *Increase in the seroprevalence due to new infections between ages a_m and a_n, $\Psi(a_m, a_n)$, obtained from the seroprevalence curve.*

Age (years)	Estimated seroprevalence, $S(a)$	$\Psi(a_m, a_n)$
10	0·390	
15	0·528	0·138
20	0·605	0·078
25	0·648	0·043
30	0·672	0·024
35	0·685	0·013
40	0·693	0·007

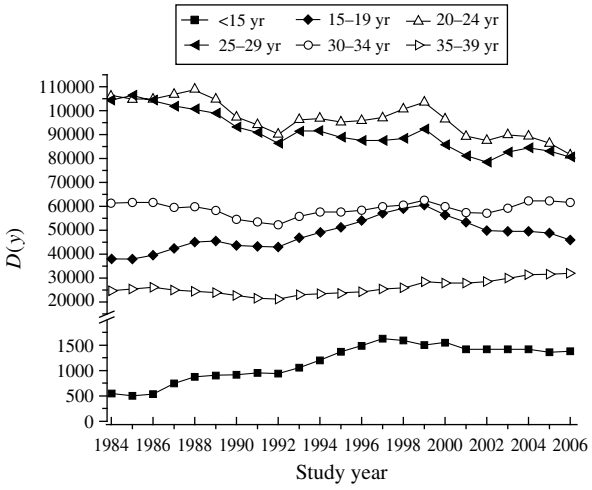

Fig. 3. Number of deliveries for each parturient age group per year y, $D(y)$, from 1984 to 2006, São Paulo Metropolitan Region, Brazil.

presents the number of deliveries for each maternal age group per year y, $D(y)$. It can be observed there was an increase in the number of births in teenagers since 1984. Figure 4 shows the estimated number of congenital toxoplasmosis cases per year $C(a_m, a_n; y)$ for each parturient age group, over the 23 years studied.

The estimated annual incidence of congenital toxoplasmosis $E(y)$ in SPMR varied from 9·5 to 10·6 cases/1000 births in the study period.

DISCUSSION

The antibody prevalence estimate for *T. gondii* in women of childbearing age obtained in this study (64·9%) is in the range of previous studies with pregnant women and parturients in São Paulo state, in

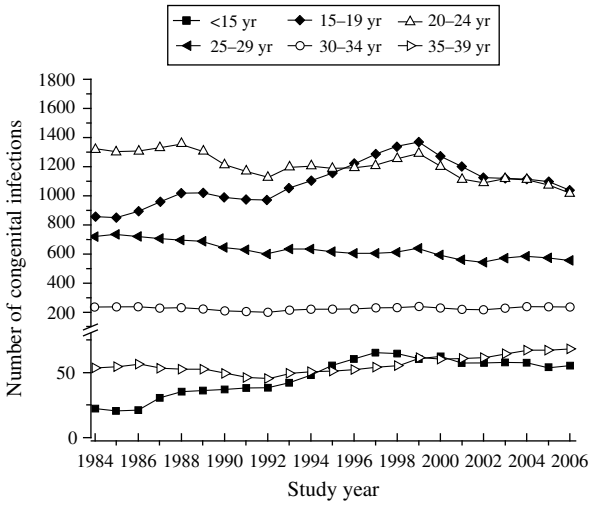

Fig. 4. Annual number of congenital infections, $C(a_m, a_n; y)$, in each parturient age group, from 1984 to 2006, São Paulo Metropolitan Region, Brazil.

which prevalence varied from 51·5% to 67·4% [31–34]. Sensitivity and specificity of assays used to estimate toxoplasmosis seroprevalence probably explains part of the variation found in studies.

The proportion of newborns with antibodies against *T. gondii* was 67%. This is in agreement with the serological findings for women of fertile age. The decay in the proportion of seropositive infants began in the first month of life, and, after 10 months of age it can be considered that maternal antibodies were cleared.

Mathematical modelling has been used previously to estimate incidence of toxoplasmosis in pregnant women. In France, from a seroprevalence of 60% for toxoplasmosis, published in previous studies, considering women aged between 15 and 44 years, Papoz *et al.* [35] found 6·4 cases of congenital toxoplasmosis per 1000 newborns. However, the authors felt that the risk of acquiring infection is equal for all ages, from birth to death, and the seroconversion was not stratified by age, which may explain the difference in the number of cases compared to our study. On the other hand, Nokes *et al.* in Stockholm, Sweden, also used seroprevalence data to apply a catalytic infection model, in pregnant women aged 15–45 years old stratified by age, but in a longitudinal study recorded over 3 years, which may have provided a more accurate result that the one obtained in our study [36].

Ades reviewed several studies in the UK on the estimation of primary infection during pregnancy by *T. gondii* and cytomegalovirus. He applied modelling

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on data obtained from these studies and proposed a method for calculating the number of women at risk, whereas the IgM persists during the same period of time for all. For toxoplasmosis, estimates of annual incidence cover sufficient variety, with significant heterogeneity within two extremes; however, the difference in this model and that used in our study makes it difficult to compare the results between them [37].

Finally, in Colombia, Gomez-Marin *et al*. [38], using two mathematical models previously developed [35, 37], estimated 7·1–10·6 new toxoplasmosis cases per 1000 pregnant women, rates lower than those found in our study.

Our model estimated congenital toxoplasmosis incidence between 9·5 and 10·6 cases/1000 births, a higher number when compared to some previously published data. This may reflect an overestimation of the model, although a Brazilian study demonstrated an estimate of eight cases of toxoplasmosis per 1000 live-birth infants from a public hospital [39].

The seroprevalence of toxoplasmosis in a representative community allowed us to estimate the congenital toxoplasmosis incidence in SPMR using mathematical modelling. A relatively simple model based on seroprevalence can provide coherent estimates for a congenital infection like toxoplasmosis in a given community, which is useful for health authorities in planning their actions towards prevention and control.

DECLARATION OF INTEREST

None.

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