

# Seroprevalence and risk factors for toxoplasmosis among antenatal women in London: a re-examination of risk in an ethnically diverse population

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**Background:** Primary infection with *Toxoplasma gondii* in pregnancy can result in miscarriage, hydrocephalus, cerebral calcification and chorioretinitis in the newborn. The objective of our study was to evaluate seroprevalence of and analyse risk factors for toxoplasmosis in antenatal women from 2006 to 2008 in an ethnically diverse population of Central London to re-examine the need for a screening policy. **Methods:** We performed serum IgG estimations to *T. gondii* using a commercial kit, and analysed risk factors for acquisition using a questionnaire. **Results:** Seroprevalence for *T. gondii* was 17.32% in 2610 samples tested. In all, 67.7% were of UK origin (seroprevalence: 11.9%) and were significantly non-immune to *T. gondii* (OR: 0.38, 95% CI: 0.31–0.47;  $P < 0.0001$ ). Risk factors for seroprevalence included African/Afro-Caribbean (OR: 2.67, 95% CI: 1.83–3.88;  $P < 0.001$ ; seroprevalence: 31.5%), Middle eastern (OR: 3.12, 95% CI: 1.62–5.99;  $P \leq 0.001$ ; seroprevalence: 34.8%) and mixed (OR: 1.75, 95% CI: 1.16–2.63;  $P = 0.007$ ; seroprevalence: 23.3%) ethnic groups; eating undercooked meat (OR: 1.64, 95% CI: 1.29–2.08;  $P \leq 0.001$ ; seroprevalence: 20.2%) and drinking unpasteurised milk (OR: 1.38, 95% CI: 1.01–1.88;  $P = 0.05$ ; seroprevalence: 23.1%). There was no association with pet cats or eating unpasteurised cheeses and antibody responses. **Conclusion:** Low national prevalence of toxoplasma seroconversion and congenital disease would likely not justify screening in the UK. Individual risk assessment is recommended in ethnically diverse urban areas where populations with relatively high seroprevalence and parasite-associated risk factors exist together with an indigenous population with low prevalence. One universal screening policy based on the indigenous prevalence and risk factors may not be suitable for all.

## Introduction

*Toxoplasma gondii* has a wide spectrum of prevalence across the globe, as indicated by the diverse seroprevalence of the parasite.<sup>1</sup> Primary infection of the mother during pregnancy is the pathogenic event, leading potentially to abortion, hydrocephalus, cerebral calcification and/or chorioretinitis.<sup>2,3</sup> Infection with *T. gondii* can be acquired from soil contaminated with cat faeces and by dietary habits, such as consumption of undercooked meat and un-pasteurised goat's milk. In many countries, it has declined sharply over the past three decades.<sup>4</sup>

As the rates of maternal-fetal transmission and fetal disease after primary infection appear to be constant, the incidence of infection is dependent on the generic seroprevalence—determining the susceptible population—and the frequency of risk factors for acquisition. In turn, seroprevalence is a key dictator of screening policy. The UK National Screening Committee recommends that screening for toxoplasmosis should not be offered routinely. The Committee based its recommendation on lack of evidence that antenatal screening and treatment reduces mother-to-child transmission or the complications associated with toxoplasma infection. It further states that antenatal screening based on monthly or 3-monthly re-testing of susceptible women would be labour intensive and would require substantial investment without any proven benefit. The policy remains that primary prevention of toxoplasmosis through avoidance of risk factors is a good alternative to antenatal screening and has not changed since 2001.<sup>5,6</sup>

In the last decade, the population mix of London has undergone several changes, with increased migration of persons from Europe and elsewhere because of the expansion of the European Union and for reasons of economic opportunity or conflict. This study presents recent *T. gondii* seroprevalence with an analysis of risk factors in a multi-ethnic antenatal cohort in London and the implications for antenatal screening for toxoplasmosis in such populations.

## Methods

We approached 5000 women who had attended the antenatal clinic at University College London Hospitals between 2006 and 2008 and had successfully delivered. All were sent information leaflets and requests for consent to participate in the study. Those who gave consent were sent a closed-question questionnaire regarding potential risk factors for toxoplasma seroprevalence. These included country of origin, habitation, obstetric history, socio-economic background, cat ownership and dietary habits (specifically whether drinking unpasteurised milk from any animal source or eating uncooked meats, such as pork, mutton, beef or minced meat products or unpasteurised cheeses). Ethnic origin was documented as a separate variable even if they were born in a different country. Questions regarding fruit and vegetable consumption, personal hygiene (a majority answered in the affirmative) and gardening (a majority answered in the negative) were withdrawn after piloting the questionnaire. Due to the nature of urban dwellings in Central

**Table 1** Demographic characteristics of subjects tested for serum antibody to *Toxoplasma gondii*

Characteristic	Category	No (%)	Number (%) positive for toxoplasma antibody	Odds ratio (OR; 95% CI) for toxoplasma seropositivity	P value
Age group (years) (Range, 16–49)	<34 years	1331 (51.00)	231 (17.36)	1	0.96
	≥34 years	1279 (49.00)	221 (17.28)	0.99 (0.81–1.22)	
Number of children	First pregnancy	329 (12.61)	77 (23.40)	1	0.002
	≥1	2281 (87.39)	375 (16.44)	0.64 (0.49–0.85)	
Accommodation	Flat/home owner	1664 (63.72)	253 (15.21)	1	0.001
	Tenant	433 (16.59)	95 (21.94)	1.57 (1.20–2.04)	
	Council	432 (16.55)	90 (20.83)	1.45 (1.12–1.92)	
	Other <sup>a</sup>	82 (3.14)	14 (17.07)	1.15 (0.64–2.07)	
Employment	Employed	1492 (57.16)	250 (16.76)	1	0.38
	Other <sup>b</sup>	1118 (42.84)	202 (18.07)	1.10 (0.89–1.34)	
Family income (£per annum)	>£40 000	1151 (44.10)	204 (17.72)	1	0.85
	£20 000–£40 000	622 (23.83)	108 (17.36)	0.98 (0.75–1.26)	
	< £20 000	837 (32.07)	140 (16.73)	0.93 (0.74–1.18)	
Born in the UK	No	971 (37.20)	256 (26.36)	1	<0.0001
	Yes	1639 (62.80)	196 (11.96)	0.38 (0.31–0.47)	
Education	None	189 (7.24)	32 (16.93)	1	0.46
	School level	432 (16.55)	84 (19.44)	1.18 (0.76–1.85)	
	College/university	1989 (76.21)	336 (16.89)	1.00 (0.67–1.48)	
Religion	Christian	1382 (52.95)	237 (17.15)	1	0.13
	Jewish	189 (7.24)	24 (12.70)	0.70 (0.45–1.10)	
	Hindu	37 (1.42)	3 (8.11)	0.43 (0.13–1.40)	
	Muslim	221 (8.47)	61 (27.60)	1.84 (1.33–2.55)	
	Other denominations including atheist	781 (29.92)	127 (16.26)	0.94 (0.74–1.19)	
Ethnic Origin	White Caucasian	2013 (77.13)	317 (15.75)	1	<0.001
	African/Afro-Caribbean	162 (6.21)	51 (31.48)	2.46 (1.73–3.50)	
	Indian Subcontinent	166 (6.36)	24 (14.46)	0.90 (0.58–1.42)	
	Far East	76 (2.91)	10 (13.16)	0.81 (0.41–1.59)	
	Middle East	43 (1.65)	15 (34.88)	2.87 (1.51–5.43)	
	Mixed	150 (5.75)	35 (23.33)	1.63 (1.09–2.42)	
Vegetarian	No	2372 (90.88)	422 (17.79)	1	0.04
	Yes	238 (9.12)	30 (12.61)	0.67 (0.45–0.99)	
Eat undercooked or rare meat	No	1529 (58.58)	234 (15.30)	1	0.001
	Yes	1081 (41.42)	218 (20.17)	1.34 (1.14–1.71)	
Drink unpasteurised milk	No	2324 (89.04)	386 (16.61)	1	0.006
	Yes	286 (10.96)	66 (23.08)	1.51 (1.12–2.03)	
Eat unpasteurised or soft cheeses	No	1100 (42.15)	195 (17.73)	1	0.63
	Yes	1510 (57.85)	257 (17.02)	0.95 (0.78–1.17)	
Cat owner	No	2252 (86.28)	400 (17.76)	1	0.13
	Yes	358 (13.72)	52 (14.53)	0.79 (0.58–1.08)	

a: Living with parents, students

b: Unemployed, retired, full-time mother, student

London most did not have access to gardens. The details of the questionnaire are tabulated in table 1.

From this cohort, 3058 women responded to the questionnaire and gave informed consent for their first antenatal serum samples to be tested. No information was available from those who did not give consent. Stored serum samples (stored at  $-70^{\circ}\text{C}$ ) were retrospectively tested for *T. gondii* IgG by commercial ELISA kits marketed by Launch Diagnostics, UK; 2610 stored samples were available for testing. The assay was performed according to the published protocol that accompanied the kit. Serum samples were tested in batches of 26 samples, with appropriate positive and negative controls. Standards of 15, 50 and 150 IU/ml were also included to give a semi-quantitative

assay and to differentiate positive from negative. The tests were read on a Bio Elisa Elx800 reader at wavelength 450 nm.

### Statistical Analysis

Statistical analysis was done using a software programme, STATA<sup>®</sup> version 8.

Univariate and multivariate analyses were performed using a logistic regression model; OR and 95% CI for risk factors associated with seroprevalence for toxoplasmosis were calculated. The multivariate model was based on significant variables from the univariate analysis and *a priori* risk factors such as pet

**Table 2** Multivariable analysis: logistic regression model for odds ratios for the presence of serum antibody for *T. gondii*

Variable	Category	Unadjusted odds ratios		P-value of OR	Adjusted odds ratios		LRT <sup>a</sup> P-value of OR
		OR	95% CI		OR	95% CI	
Eat undercooked or rare meat	No	1					
	Yes	1.34 (1.14–1.71)		0.001	1.64 (1.29–2.08)		<0.0001
Drink unpasteurised milk	No	1					
	Yes	1.51 (1.12–2.03)		0.006	1.38 (1.01–1.88)		0.05
Eat unpasteurised or soft cheeses	No	1					
	Yes	0.95 (0.78–1.17)		0.63	0.87 (0.68–1.13)		0.30
Cat owner	No	1					
	Yes	0.79 (0.58–1.08)		0.13	0.84 (0.61–1.16)		0.28
Ethnic Origin	White Caucasian	1					
	African/Afro-Caribbean	2.46 (1.73–3.50)		<0.001	2.67 (1.83–3.88)		<0.001
	Indian subcontinent	0.90 (0.58–1.42)		0.66	0.98 (0.62–1.57)		0.94
	Far East	0.81 (0.41–1.59)		0.54	0.82 (0.41–1.62)		0.56
	Middle East	2.87 (1.51–5.43)		0.001	3.12 (1.62–5.99)		0.001
	Mixed	1.63 (1.09–2.42)		0.02	1.75 (1.16–2.63)		0.007

a: Likelihood ratio test

n=2610, only cases with no missing values were analysed

ownership. The adequacy of the model was examined by post-estimation goodness-of-fit tests, namely the Hosmer–Lemeshow test.

Power calculations were done using Epi-Info software, and the study sample size achieved 90% power at the 5% level for an OR of 2.0.

## Results

### Overall positivity for *T. gondii* IgG was 17.32%

Over 110 countries were represented as country of origin. A major proportion of these subjects, 1639 (63%) were born in the UK. Bangladeshi origin was the next most common, accounting for 58 (2.2%). France and the United States each accounted for 54 (2.1%). The Mediterranean Europe and Northern Europe countries were represented in 194 (7.4%) and 243 (9.3%) subjects, respectively. This represented a significant ( $P=0.02$ ) north–south difference in distribution. There was no significant difference in the prevalence of toxoplasmosis between the Northern (15.1%) and Southern/Mediterranean European countries (15.6%). The Middle Eastern countries were represented by Israel, Bahrain, Dubai, Saudi Arabia, Iraq, Iran and Lebanon ( $n=30$ , 0.11%). Continents were represented as follows: Europe excluding the UK 461 (17.65%); Asia 151 (5.8%); Africa 118 (4.5%); Australasia 76 (2.9%); North America 71 (2.7%); South America 52 (2.0%) and Oceania 1 (0.04%); 41 (1.6%) declined to answer this question. The demographic profile showed that 51% were <34 years of age and 88% had one or more child/children. In this cohort, 64% were home owners, 17% lived in rented property and 17% in council housing.

Demographic characteristics of our client population and univariate analysis are described in table 1.

By univariate analysis the following social factors were observed as risk factors for seropositivity: property tenant (OR: 1.57, 95% CI: 1.20–2.04;  $P=0.001$ ); Afro-Caribbean origin (OR: 2.46, 95% CI: 1.73–3.50;  $P<0.001$ ); Middle Eastern origin (OR: 2.87, 95% CI: 1.51–5.43;  $P=0.001$ ) and mixed origin (OR: 1.63, 95% CI: 1.09–2.42;  $P=0.02$ ); Muslim faith (OR: 1.84, 95% CI: 1.33–2.55;  $P<0.001$ ).

The odds of being antibody positive were less for those born in UK (OR: 0.38, 95% CI: 0.31–0.47;  $P<0.0001$ ) and those having one or more child/children (OR: 0.64, 95% CI: 0.49–0.85;  $P=0.002$ ).

In terms of dietary characteristics, those who ate undercooked or rare meat (OR: 1.34, 95% CI: 1.14–1.71;  $P=0.001$ ) and those drinking unpasteurised milk (OR: 1.51, 95% CI: 1.12–2.03;  $P=0.006$ ) were more likely to be seropositive.

A multivariate model was constructed using relevant significant variables from the univariate analysis and *a priori* risk factors for toxoplasma seropositivity, such as cat ownership and dietary characteristics (table 2). Since ethnic origin, country of birth and religion had significant co-linearity only ethnic origin was included in the model. This table shows that the presence of antibody was associated with the African/Afro-Caribbean (adjusted OR: 2.67, 95% CI: 1.83–3.88;  $P<0.001$ ), Middle eastern (adjusted OR: 3.12, 95% CI: 1.62–5.99;  $P\leq 0.001$ ) and mixed; such as of British-African or Eurasian parentage (adjusted OR: 1.75, 95% CI: 1.16–2.63;  $P=0.007$ ) ethnic groups; eating undercooked meat (adjusted OR: 1.64, 95% CI: 1.29–2.08;  $P\leq 0.001$ ); and drinking unpasteurised milk (adjusted OR: 1.38, 95% CI: 1.01–1.88;  $P=0.05$ ). There was no association with employment status, family income, educational background, owning pet cats or eating unpasteurised cheeses and a positive antibody response.

## Discussion

*Toxoplasma gondii* seroprevalence varies widely in different parts of the world. Pappas *et al.*, in 2009, published a review of global seroprevalence and its implications for pregnancy.<sup>1</sup> A study from the USA showed that seroprevalence in the childbearing age was 11%.<sup>7</sup> As in our study, they showed that the seroprevalence was 7.7% for women born in the USA and 28.1% in those who were foreign-born. In the South American and Caribbean countries, the seroprevalence varied from 40–70%.<sup>1</sup> The authors' review of European studies on toxoplasma seroprevalence in pregnancy or in females of childbearing age confirms that they do not homogeneously depict the general European seroprevalence status, with certain countries such as Greece being over-represented. In general, the seroprevalence varied from 20–40%. High seroprevalence was found in Belgium,<sup>8</sup> Germany<sup>9</sup> and Poland<sup>10</sup> (in excess of 40%). Low prevalence (9.1%) was documented in a UK study from 2005.<sup>11</sup> This figure is similar to the seroprevalence of our UK-born subjects rather than the overall seropositivity rate. Nash *et al.* concluded that prevalence was related to rural or continental childhood residence.<sup>11</sup>

Pappas *et al.* commented on high prevalence of foci in the Middle East including Turkey, Iran, Iraq and Kuwait.<sup>1</sup> A general population study in Eastern Saudi Arabia<sup>12</sup> showed a lower prevalence, reaching 25%, similar to pregnant women in Bahrain<sup>13</sup> and to the general population prevalence observed in Qatar.<sup>14</sup> In Asia, high rates were found in Indonesia (>60%)<sup>15</sup> and Malaysia (49%)<sup>16</sup> but significantly lower rates in China<sup>17</sup> and Vietnam<sup>18</sup> (10–11%). In India, most studies showed a rate in excess of 40%.<sup>19–21</sup> The few studies quoted from Africa showed prevalences from 25% in Burkina

Faso<sup>22</sup> to 60% in the Ivory Coast.<sup>23</sup> One Australian study documented a seroprevalence of 23%.<sup>24</sup>

Pappas *et al* make the observation that the cumulative results of their review indicate that seroprevalence rates are evolving through time in a varying manner: a general trend towards lower rates is observed in the Western world, but concern should be raised by the potential effect of the increasing influx of immigrants from developing *T. gondii* endemic countries into the industrialised world. They recommend monitoring seroprevalence since population influx may continuously alter local seroprevalence rates in the developed world.<sup>1</sup>

The results of our study indicate that the risk factors for antibody prevalence in the antenatal population from Central London were related to being non-UK born, particularly of African/Afro-Caribbean, Middle Eastern and mixed origins. A Norwegian study has found higher seroprevalence in women with foreign names<sup>25</sup> and association with country of birth and ethnic group was reported in a study of pregnant women in London.<sup>26</sup>

Association of seropositivity with undercooked meat has been repeatedly observed in the past.<sup>25,27–33</sup> This is seen across studies of different population types. Elsheikha *et al* studying Egyptian blood donors (total n = 260) showed significant risk factors for seropositivity with eating luncheon/shawarma meat.<sup>30</sup>

With regard to our population of interest—women in the reproductive age group—Bobic *et al* found, in a study of 1157 patients, that toxoplasma infection was associated with consumption of undercooked meat. This association was greater in women living in suburbs compared with central urban zones. Soil contact (by either gardening or farming) was significant for women aged 15–19 years.<sup>28</sup> We elected not to include gardening as a risk factor because of the urban nature of a large proportion of dwellings in Central London (flats that do not have access to gardens).

Baril *et al* conducted a case-controlled study of 80 non-immune women who seroconverted to toxoplasma positive during pregnancy. By multivariate analysis, risk factors with significant odds ratios for toxoplasma seroconversion were poor hand hygiene (an amalgam of variables ‘no hand-washing before meals’ and ‘no hand-washing after food preparation’) consumption of undercooked beef and lamb, consumption of raw vegetables outside the home and ownership of a pet cat. No significant associations were observed with eating unpasteurised cheese or undercooked eggs. In contrast, other studies including ours showed no association with cat ownership. After piloting our questionnaire, we decided to withdraw questions on fruit and vegetable consumption and personal hygiene, as we had a majority affirmative response and could not rule out responder bias.

A prospective Norwegian study on pregnant women with recent seroconversion found significant association with eating raw or undercooked minced meat products, unwashed raw fruits and vegetables and raw or undercooked mutton and pork. Significant association was also seen with cleaning a cat litter box and infrequent washing of knives between preparing raw meat and preparing other food. This study involved 63 pregnant women with 128 controls matched by age, state of pregnancy, expected date of delivery and geographical area.<sup>25</sup> The study illustrates the less often reported risk factors of cross-contamination of knives in food preparation and the risk of inoculation injury to pregnant women.

In our study, age was not a risk factor for seroconversion. Other studies have reported that older age groups are more likely to have seroconverted,<sup>11,28</sup> whereas some researchers have found that women <25 years of age had a high seroprevalence of toxoplasma IgG.<sup>21</sup> It is not clear why age was not a significant risk factor for toxoplasmosis in our study. It is probably because our subjects came from diverse backgrounds, with varied dietary and travel/migration history to the UK, which could have been influenced by individual circumstances, thus resulting in different ages of exposure to the parasite. Furthermore, our study was a prevalence-based study and did not

measure age at seroconversion, and hence the cross-sectional design is a limitation in analysing the influence of age on seroconversion.

Risk associations of poor hygiene practices<sup>25,33</sup> and cat contact<sup>25,27,31,34</sup> are inconsistently observed worldwide and likely in part to be due to variable human behaviours and local customs. Given the spectrum of potential host–parasite interactions, other significant sources/behaviours may yet be elucidated by larger studies.

The question of whether to screen for antenatal toxoplasmosis in the UK is highly controversial. In many developed countries, including the UK, the low probability of congenital infection coupled with the associated low positive predictive value of serology to detect genuine infection has stood against screening.<sup>35</sup> In the setting of low prevalence, false-positive diagnoses of congenital toxoplasmosis can cause anxiety and may even lead to unnecessary termination of pregnancy, whereas false-negatives generate false reassurance. Conversely, countries that practice antenatal screening such as France do so on the basis of assessed benefit analysis; the supporting data depend on the effect of a high parasite prevalence—giving a high probability of relatively few seronegative individuals acquiring infection. That said, randomized control trials indicating objective benefit of treatment outcomes of screening vs. no screening strategies are lacking. Despite the many studies cataloguing generic and acute seroprevalence, no systematic correlation is made between prevalence rates and their attributable risk to congenital infection.<sup>36,37</sup> One foreseeable problem with instigating routine IgG testing in pregnancy is that of repeat screening. A seronegative result in early pregnancy would leave patient and clinician in a quandary later in pregnancy as to whether infection had or had not occurred subsequently. Hence, a dilemma as to when further seroconversion checks should be done. Ideally, seroconversion status should be ascertained prior to pregnancy, and if seronegative, clients need to be checked at regular intervals during pregnancy with the obvious disadvantages of generating anxiety and misinterpretation of test results. At present, the low national prevalence of toxoplasma seroconversion and congenital disease would likely not justify screening in the UK. However, the situation within metropolitan zones, (where populations with relatively high seroprevalence and parasite-associated risk factors exist) merits close vigilance. This is because residents of large metropolises such as London are exposed to diverse and authentic cuisines from around the globe.

Our study is generalisable to large urban metropolises that are home to multi-ethnic populations similar to Central London: UK-born individuals had low seroprevalence as reported by one other study<sup>11</sup>; our White Caucasian subjects reflected the seroprevalence found in the industrialised nations; high seroprevalence in the African/Afro-Caribbean, Middle Eastern and mixed cohort is similar to the global data reviewed by Pappas *et al*.<sup>1</sup>

A limitation of our study is that we had no information on those who did not give consent (n = 1942; 39%); however, this was overcome by the sample size that we were able to recruit into the study. For the major variables tested for risk, we had sufficient sample numbers to achieve 80% power at 5% significance level. We were able to analyse 2610 (85%) of the samples provided by the 3058 women who gave consent. The samples that were not tested had insufficient serum and this occurred in a random manner, and therefore did not cause bias. Statistical goodness-of-fit estimations showed that the model was a good fit.

In conclusion, all antenatal women should be made aware of the risks of eating undercooked meats (beef or mutton), drinking unpasteurised milk especially goat’s milk, consuming unwashed raw fruits and vegetables, using the same utensils for raw meat and other prepared foods, inoculation injuries while chopping raw meat and gardening without wearing gloves. Although it is acceptable to withhold screening in countries like the UK where there is low prevalence of congenital infection, it is important to bear in mind that this may not be applicable universally to

multi-ethnic communities in large metropolitan cities such as London. We favour an individual risk assessment of non-indigenous clients in the UK, for exposure to risks such as drinking unpasteurised milk and eating undercooked meats, and to offer them toxoplasma screening in pregnancy.

*Conflicts of interest:* None declared.

## Key points

- The parasite *Toxoplasma gondii* has the potential to cause severe congenital disease, if primary infection is acquired during pregnancy.
- The low overall seroprevalence rate in the UK does not justify screening of all pregnant women.
- Seroprevalence is significantly higher in non-UK-born women.
- In multi-ethnic metropolitan areas, where a combination of populations with high toxoplasma prevalence and risk behaviours exist, an individual risk assessment is favoured and toxoplasma screening is justified according to risk.

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