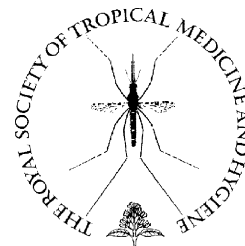




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Serological screening and toxoplasmosis exposure factors among pregnant women in the Democratic Republic of Sao Tome and Principe

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Summary The seroprevalence of *Toxoplasma gondii* infection among pregnant women in the Democratic Republic of Sao Tome and Principe (DRSTP) from November 2003 to March 2004 was determined by detection of serum anti-*T. gondii* antibodies. A short questionnaire interview for pregnant women was performed to investigate risk factors associated with *T. gondii* infection, including consumption of raw meat or unwashed vegetables, drinking unboiled water and keeping pets (cats and dogs). The overall seroprevalence of *T. gondii* infection was high (75.2%; 375/499). The older age group of ≥ 35 years had a significantly higher seroprevalence (85.7%; 54/63) than that of the younger age group of 15–25 years (70.4%; 178/253) (odds ratio 2.5, 95% CI 1.2–5.4; $P=0.01$). No significant difference in the seroprevalence of *T. gondii* infection was found between the pregnant women with and without exposure to the risk factors studied. However, among pregnant women with high antibody titers of $\geq 1:1024$, it seemed likely that

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continual contact with pets and consumption of oocyst-contaminated water or raw unwashed vegetables rather than tissue cysts in meat was the primary route of infection. The incidence of congenital toxoplasmosis in unborn babies should be intensively monitored in the DRSTP.

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1. Introduction

Toxoplasma gondii is a protozoan parasite that is endemic worldwide and is a major opportunistic pathogen in immunocompromised hosts. Infection is mainly acquired by ingestion of food, water or soil that is contaminated with oocysts shed by cats, or by eating undercooked or raw meat containing tissue cysts (Fayer et al., 2004). Primary infection is usually subclinical, but in severely immunocompromised patients it may be life-threatening, i.e. encephalitis, or the greater threat of a latent infection is increasingly likely due to reactivation as immune function decreases (Montoya and Liesenfeld, 2004). Newly acquired *T. gondii* infection in a pregnant woman can be transmitted to the fetus and may cause cognitive impairment, blindness, epilepsy and death. Although congenital toxoplasmosis may be asymptomatic at birth, ocular problems may manifest later in life (Montoya and Liesenfeld, 2004). For the diagnosis of *T. gondii* infection, detection of the organism itself is confirmative but very difficult. Thus, most clinical laboratories use serological tests to detect antibodies against *T. gondii*, such as the latex agglutination (LA) test, ELISA and indirect fluorescent antibody test. The LA test has been widely used to screen for *T. gondii* infection because of its high specificity and high sensitivity (Fan et al., 2001, 2002, 2003, 2006; Fleck, 1989; Woldemichael et al., 1998).

In developed countries, congenital toxoplasmosis affects between 1 and 10 in 10 000 newborn babies (Allain et al., 1998; Gilbert and Peckham, 2001). In Brazil, the incidence of congenital toxoplasmosis was estimated approximately to be 1 in 4800 newborn babies (Neto et al., 2000). Effective prevention of congenital toxoplasmosis depends on avoidance of infection during pregnancy (Boyer et al., 2005). Serological screening for *T. gondii* antibodies should therefore be done in pregnant women because absence of IgG antibodies before or early in pregnancy allows identification of women at risk of acquiring the infection (Montoya and Liesenfeld, 2004). In Europe, the prevalence of *Toxoplasma* infection among pregnant women ranges from 7.7 to 45.0% (Allain et al., 1998; Jennum et al., 1998; Logar et al., 1995). In Africa, few reports concerning the seroprevalence of *T. gondii* infection in pregnant women in Western and Eastern Africa countries are available, e.g. 78% (273/352) in Nigeria, 53.6% (113/211) in Benin (Onadoko et al., 1992; Rodier et al., 1995); the status of *T. gondii* infection among pregnant women in the Democratic Republic of Sao Tome and Principe (DRSTP) is unknown to date.

Our previous study in the DRSTP indicated that the seroprevalence of *T. gondii* infection among pre-schoolchildren aged under 5 years was fairly high (21.49%, 26/121), and it seemed that consumption of oocysts shed from domestic or stray cats, contaminated soil or water was a possible route of infection for children (Fan et al., 2006). To determine the risk factors for toxoplasmosis exposure, a

seroepidemiological survey of *T. gondii* infection among pregnant women in Sao Tome Island of the DRSTP using the LA test was conducted from November 2003 to March 2004. Additionally, a questionnaire interview to determine personal details, including each study individual's history of exposure to risk factors associated with infection, was included.

2. Materials and methods

2.1. Study population and subject selection

The DRSTP consists of the two islands of Sao Tome and Principe and a number of smaller islets in the Gulf of Guinea. Sao Tome lies approximately 180 miles from the coast of Gabon and is crossed by the equator at its southern tip. The climate is tropical with two rainy seasons. The total number of inhabitants of the DRSTP is estimated to be 160 000, and the total number of inhabitants of Sao Tome Island is approximately 150 000.

Pregnant women (mean age \pm SD: 25.4 \pm 6.4 years) who visited the National Center for Health Care of Women and Children on Sao Tome Island were invited to participate in the present study after giving their oral informed consent. Serum samples were obtained by venipuncture and were kept at -20°C at the Medical Mission of Taiwan in the DRSTP until use.

2.2. Ethical approval

Ethical approval for the study was obtained from the Ministry of Hygiene of the Democratic Republic of Sao Tome and Principe.

2.3. Serological detection of *Toxoplasma gondii* infection by latex agglutination test

Sera obtained from pregnant women during their first trimester (Gilbert and Peckham, 2001) were screened for *T. gondii* antibodies using the *Toxoplasma* latex agglutination test (TOXO Test-MT, Eiken Co. Ltd, Tokyo, Japan) by following the manufacturer's instructions. Titers of ≥ 32 (i.e. 1:32 to 1:2048) were regarded as positive (Fan et al., 2001, 2006). Compared to the Sabin-Feldman test, the sensitivity and specificity of TOXO Test-MT were 96.3% and 97.1%, respectively (Woldemichael et al., 1998).

2.4. Questionnaire interview on *Toxoplasma gondii* infection

A short questionnaire interview for pregnant women was carried out to obtain details of factors associated with *T. gondii* infection, including age, residential district, history

of eating raw meat or unwashed vegetables, drinking unboiled water and keeping pets including cats and/or dogs.

2.5. Statistical analysis

The subjects were categorized into three age groups (15–25 years; 25–35 years; ≥ 35 years). Statistical analysis was performed using SAS software (SAS Institute Inc., Cary, NC, USA). The increasing trends of age-specific seropositive rates were tested for statistical significance using the χ^2 test for trends. Risk factor (RF) assessment between pregnant women with and without histories of exposure to eating raw meat, unwashed vegetables, drinking unboiled water or keeping pets was tested for statistical significance; if the difference was insignificant, further RF assessment was performed among seropositive pregnant women with high LA titers to find the possible source of *T. gondii* infection. Crude odds ratios with their 95% CIs were estimated by means of multiple logistic regression analysis. The statistical significance of differences in variables associated with seropositivity for *T. gondii* infection among comparison groups was examined by testing the statistical significance of regression coefficients. *P*-values < 0.05 were considered to be significant.

3. Results

Of the 499 serum samples studied, 375 (75.2%) were positive for *Toxoplasma* antibodies and the seroprevalence increased with age (Table 1). Interestingly, 61.3% (230/375) of the pregnant women had high LA titers of $\geq 1:1024$. The seropositivity for *T. gondii* infection in those women with and without histories of eating raw meat or unwashed vegetables, drinking unboiled water or keeping pets is shown in Table 1. Further analysis of the questionnaire data revealed that, of the 230 pregnant women with an LA titer of $\geq 1:1024$, 1.0% (2/204) had a history of eating raw meat compared to 38.4% (76/198) for keeping pets, 72.6% (148/204) for eating unwashed vegetables and 89.4% (186/208) for drinking unboiled water (Table 1).

Multiple logistic regression analysis revealed that women aged 25–35 years and ≥ 35 years had a higher risk for *T. gondii* infection when compared to those aged 15–25 years ($P=0.07$, $P=0.01$, respectively) (Table 1). Those who had histories of eating raw meat ($P=0.24$) or unwashed vegetables ($P=0.21$), drinking unboiled water ($P=0.91$) or keeping pets ($P=0.34$) had higher *Toxoplasma* seroprevalence when compared with those without such a history. However, 230 pregnant women with an LA titer of $\geq 1:1024$ with a history of keeping pets, eating unwashed vegetables or drinking unboiled water had a higher risk for *T. gondii* infection when compared to those with a history of eating raw meat ($P < 0.001$).

4. Discussion

The present study indicates that the overall seroprevalence of *T. gondii* infection among pregnant women in DRSTP is high (75.2%, 375/499), and is significantly higher than that of pregnant women in Taiwan (10.2%, 183/1796; Yu, 1985)

and Korea (4.3%, 39/899; Ryu et al., 1996) in Asia, Norway (10.9%, 3907/35 940; Jenum et al., 1998) and Slovenia (45.0%, 9430/20 953; Logar et al., 1995) in Europe, and Benin (53.6%, 113/211; Rodier et al., 1995) in Eastern Africa, whereas it is similar to that of pregnant women in Nigeria (78.0%, 273/352; Onadoko et al., 1992) in Western Africa. The discrepant seroprevalence between countries might be explained by differences in socio-economic status and food habits, e.g. Asian pregnant women consumed well-cooked food and clean water (Ryu et al., 1996; Yu, 1985), while consumption of inadequately cooked or cured meat was frequently found among pregnant European women (Cook et al., 2000). However, contact with an environment contaminated by cat feces was the primary source of infection for pregnant Nigerian women (Onadoko et al., 1992).

High LA titers of anti-*Toxoplasma* antibodies might be regarded as either acute infection (Kook et al., 1999; Mackie et al., 1971) or reinfections (Onadoko et al., 1992). In the present study, most of the seropositive women (61.3%, 230/375) had high *Toxoplasma* LA titers ($\geq 1:1024$). If these women were to be infected with *T. gondii* during the first trimester, it could be very harmful to the fetus because after maternal acquisition of *T. gondii* for the first time during gestation, the parasite will enter the fetal circulation by infection of the placenta. This may result in severe congenital toxoplasmosis and can result in death of the fetus in utero and spontaneous abortion (Montoya and Liesenfeld, 2004). However, in this study it was regrettable that sera IgM or IgG avidity were not examined and thus it was unclear whether the seropositive cases with high LA titers had been infected in the past 6 months.

Alternatively, reinfections may occur frequently due to continual exposure to the risk factors; consumption of uncooked foods, water or keeping pets including dogs and cats was commonly found among DRSTP pregnant women with high LA titers thus resulting in relatively high antibody titers being maintained in this population. They might also have been exposed to the risk factors since childhood. This possibility is supported by the finding that *T. gondii* infection was fairly prevalent in DRSTP pre-schoolchildren aged < 5 years (Fan et al., 2006). Another explanation is that DRSTP women on farms spend more time exposed to *T. gondii*, since they carry out activities related to farming and thus have a higher probability of coming into contact with oocysts.

On the other hand, among the study women, 24.8% (124/499) did not present specific antibodies against *T. gondii*, so this group of women was highly likely to be susceptible to first infection during pregnancy and possible occurrence of congenital transmission due to dietary conditions and contact with environments contaminated by oocysts. Therefore, there was an urgent need for them to avoid being infected by *T. gondii* to decrease risks of transplacental transmission during gestation (Montoya and Liesenfeld, 2004).

In the present study, although no significant difference in seroprevalence of *T. gondii* infection was found between the pregnant women with and without histories of exposure to the risk factors considered, high seropositive rates in both groups suggest that they were equally at risk of being infected. Nevertheless, consumption of oocyst-contaminated water, vegetables or soil rather than tissue cysts in meats was the primary route of infection for the

Table 1 Seroprevalence, latex agglutination (LA) titer, and crude odds ratios (OR) with 95% CIs for various risk factors associated with seropositivity of *Toxoplasma gondii* antibody among pregnant women in the Democratic Republic of Sao Tome and Principe

Variable	No. tested	No. positive (%)	LA titer							OR	95% CI	P-value
			1:32	1:64	1:128	1:256	1:512	1:1024	1:2048			
Age group (years)												
15–25	253	178 (70.4)	3	6	15	16	24	44	70	Ref.	—	—
25–35	183	143 (78.1)	2	5	8	15	23	35	55	1.5	1.0–2.3	0.07
≥35	63	54 (85.7)	3	4	5	10	6	6	20	2.5	1.2–5.4	0.01
Risk factors												
Eating raw meat												
No	436	327 (75.0)	—	—	—	—	—	—	—	Ref.	—	—
Yes	42	28 (66.7)	—	—	—	—	—	—	—	0.7	0.3–1.3	0.24
Eating unwashed vegetables												
No	115	91 (79.1)	—	—	—	—	—	—	—	Ref.	—	—
Yes	367	269 (73.3)	—	—	—	—	—	—	—	0.7	0.4–1.2	0.21
Drinking unboiled water												
No	71	53 (74.7)	—	—	—	—	—	—	—	Ref.	—	—
Yes	423	313 (74.5)	—	—	—	—	—	—	—	1.0	0.5–1.7	0.91
Keeping pets												
No	271	205 (75.7)	—	—	—	—	—	—	—	Ref.	—	—
Yes	176	126 (71.6)	—	—	—	—	—	—	—	0.8	0.5–1.3	0.34
Risk factor assessment among pregnant women with LA titer of ≥1:1024												
Eating raw meat	204	2 (1.0)	0	0	0	0	0	1	1	Ref.	—	—
Keeping pets	198	76 (38.4)	0	0	0	0	0	24	52	62.9	15.2–260.8	<0.001
Eating unwashed vegetables	204	148 (72.6)	0	0	0	0	0	58	90	266.9	64.1–1111.3	<0.001
Drinking unboiled water	208	186 (89.4)	0	0	0	0	0	64	122	853.9	198.1–3681.3	<0.001
Total	499	375 (75.2)	8	15	28	41	53	85	145	—	—	—

Ref.: referent.

seropositive pregnant women. This assumption was supported by the fact that drinking unboiled water, eating unwashed vegetables, or keeping pets had relatively higher risks than eating undercooked meats in the acquisition of *T. gondii* infection as revealed by analyzing questionnaire data among pregnant women with high LA titers of $\geq 1:1024$. Meat consumption in the DRSTP people is infrequent due to economic problems (Fan et al., 2006), and given the abundance of cats (both domestic and stray) (Fan et al., 2006) and the suitable climatic conditions for sporulation of *Toxoplasma* oocysts in tropical regions (Frenkel et al., 1975), it seems likely that exposure to environments and foods contaminated by oocysts shed from cat's feces is one of the most important factors associated with *Toxoplasma*. This situation is similar to that in Nigeria where contact with cat feces was the primary source of infection among pregnant women (Onadeko et al., 1992).

The findings of increasing seropositivity rates of toxoplasmosis with age among pregnant women were similar to those seen in studies conducted in various countries (Hill and Dubey, 2002; Morris and Croxson, 2004; Petersson et al., 2000). A possible explanation for this might be their longer exposure to the risk factors related to *T. gondii* infection.

In conclusion, our study demonstrates that *Toxoplasma* infection is highly prevalent in pregnant women in the DRSTP. Our findings suggest that improvements in hygiene and sanitation are urgently needed to lower the risk of *Toxoplasma* infection in the DRSTP, and further attention should be paid to whether a high incidence of congenital toxoplasmosis is present in newborn infants in the DRSTP.

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

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