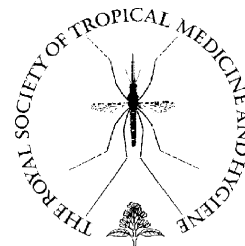




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Seroprevalence of *Toxoplasma gondii* infection among pre-schoolchildren aged 1–5 years in the Democratic Republic of Sao Tome and Principe, Western Africa

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Summary The prevalence status of *Toxoplasma gondii* infection in children of the Democratic Republic of Sao Tome and Principe (DRSTP), Western Africa, is unknown to date. A serologic survey of *T. gondii* infection among pre-schoolchildren aged <5 years in the DRSTP was assessed by the latex agglutination (LA) test from November 2003 to March 2004. The overall seroprevalence of *T. gondii* infection was not low, reaching 21.49% (26/121). No significant gender difference in seroprevalence was found between boys (19.30%; 11/57) and girls (23.44%; 15/64) ($\chi^2 = 0.31$, $P = 0.58$). The older age group of 4–5 years had significantly higher seroprevalence (36.67%; 11/30) than the younger age group of <2 years (10.34%; 3/29) ($\chi^2 = 5.64$, $P = 0.02$). It was noteworthy that the majority of seropositive boys (90.91%; 10/11) or older children aged ≥ 2 years (82.61%; 19/23) had high LA titres of $\geq 1:1024$, indirectly indicating acute *Toxoplasma* infection. This study is the first report indicating that *T. gondii* infection is not low in

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pre-schoolchildren aged <5 years in the DRSTP. Whether the DRSTP pre-schoolchildren acquire *T. gondii* infection through constant exposure to the parasite from their daily activities owing to poor environmental hygiene should be further evaluated.

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1. Introduction

Toxoplasma gondii is horizontally transmitted to humans by accidental ingestion of oocysts in water, food or soil contaminated with cat's faeces, or by eating raw or undercooked meat containing cysts (Fayer et al., 2004). Newly-acquired *T. gondii* infection in a pregnant woman can be transmitted to the fetus and may cause mental retardation, blindness, epilepsy and death. Although congenital toxoplasmosis may be asymptomatic at birth, ocular problems may manifest later in life (Montoya and Liesenfeld, 2004). *Toxoplasma gondii* can also cause severe encephalitis via acute infection or reactivation of latent infection among immunosuppressed persons. Although toxoplasmosis is the most frequent severe neurological infection among persons with AIDS, adults and children with normal immune function are usually asymptomatic or have symptoms such as fever, malaise and lymphadenopathy that resolve spontaneously (Montoya and Liesenfeld, 2004).

Infection by the protozoan parasite *T. gondii* is widely prevalent in animals and humans worldwide (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 IFAT. The LA test has been widely used to screen for *T. gondii* infection owing to its high specificity and sensitivity (Woldemichael et al., 1998).

In developed countries, the prevalence of *Toxoplasma* infection among pre-schoolchildren was low, with a range from 0.0% in Japan to 6.3% in Ireland (Suzuki et al., 1988; Taylor et al., 1997). In Asia and Latin America, the seroprevalence in Iran and Bolivia was quite high, reaching 30.7% and 47.8%, respectively (Ghorbani et al., 1978; Paradisi et al., 1989). In Africa, only one study regarding the seroprevalence of *T. gondii* infection in pre-schoolchildren of Somalia was available (Ahmed et al., 1988); information relating to *T. gondii* infection among pre-schoolchildren in the Democratic Republic of Sao Tome and Principe (DRSTP) is unknown to date.

In this study, we examined *T. gondii* antibody titres using the LA test of 121 pre-schoolchildren <5 years of age who were admitted to the National Center for Health Care of Women and Children in the DRSTP for a routine health check-up, i.e. weight, height and body temperature, from November 2003 to March 2004.

2. Materials and methods

2.1. Study population and subject selection

The DRSTP consists of 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 Gabon on the West

African coast and is crossed by the equator at its southern tip. The climate is tropical with two rainy seasons. The total number of inhabitants in the DRSTP is estimated to be 160 000, and the total number of inhabitants in Sao Tome Island is approximately 150 000.

Pre-schoolchildren (mean age \pm SD, 36.61 \pm 15.36 months) who lived on Sao Tome Island participated in the present study after informed consent was obtained from their parents or guardians. In total, 121 serum samples (57 from boys and 64 from girls) were randomly collected by venipuncture from apparently healthy pre-schoolchildren. The mean ages were similar for both genders and ranged between 1 year and 4 years for all pre-schoolchildren. All serum specimens were kept at -20°C at the Medical Mission of Taiwan in the DRSTP until laboratory examination.

2.2. Ethical approval

Ethical approval for the study was obtained from the Ministry of Hygiene of the DRSTP.

2.3. Serological detection of *T. gondii* infection by LA test

In this study, all sera were screened for *T. gondii* infection using *Toxoplasma* LA test (TOXO Test-MT; Eiken Co. Ltd, Tokyo, Japan) following the manufacturer's instructions. Titres of tested sera ≥ 32 (i.e. 1:32 to 1:2048) were regarded as positive. Compared with the Sabin–Feldman test, the sensitivity and specificity of the TOXO Test-MT was 96.3% and 97.1%, respectively (Woldemichael et al., 1998).

2.4. Statistical analysis

In the present study, subjects were categorised into four age groups (<2 years, 2–3 years, 3–4 years and 4–5 years). Statistical analysis was performed using the SAS software system (SAS Institute Inc., Cary, NC, USA). Comparison of groups was done using the χ^2 test, and *P*-values less than 0.05 were considered to be statistically significant.

3. Results

Of the total 121 serum samples studied, 21.49% (26/121) were positive for *Toxoplasma* antibody (Table 1). Although the seroprevalence of 23.44% (15/64) in girls was higher than the seroprevalence of 19.30% (11/57) in boys, this difference was insignificant ($\chi^2 = 0.31$, *P* = 0.58) (Table 1). Nevertheless, the majority of seropositive boys (90.91%; 10/11) had high LA titres of $\geq 1:1024$, whereas only 9 of 15 (60%) seropositive girls did (Table 1). Seroprevalence tended to increase with age; seroprevalence was highest in the age group 4–5 years (36.67%; 11/30), followed by the age group

Table 1 Seroprevalence of *Toxoplasma gondii* infection and latex agglutination (LA) titres among pre-schoolchildren aged <5 years in the Democratic Republic of Sao Tome and Principe, Western Africa

Variable	Group	No. tested	No. positive (%)	LA titre							χ^2	P-value	
				1:32	1:64	1:128	1:256	1:512	1:1024	1:2048			
Gender	Boys	57	11 (19.30)	0	0	0	0	1	1	1	9	Referent	—
	Girls	64	15 (23.44)	2	1	2	1	0	1	1	8	0.31	0.58
Age group (years)	<2	29	3 (10.34)	1	0	1	1	0	0	0	0	Referent	—
	2–3	26	4 (15.38)	1	0	0	0	0	1	1	2	0.31	0.58
	3–4	36	8 (22.22)	0	1	0	0	1	0	0	6	1.61	0.20
	4–5	30	11 (36.67)	0	0	1	0	0	1	1	9	5.64	0.02
Total		121	26 (21.49)	2	1	2	1	1	2	2	17	—	—

3–4 years (22.22%; 8/36), 2–3 years (15.38%; 4/26) and <2 years (10.34%, 3/29) (Table 1). Interestingly, the majority (82.61%, 19/23) of children aged ≥ 2 years had high LA titres of $\geq 1:1024$; in contrast, all of the three seropositive children aged <2 years had low LA titres of $\leq 1:256$ (Table 1). In addition, pre-schoolchildren aged ≥ 4 years had a significantly higher seroprevalence of *T. gondii* infection compared with those aged <2 years ($\chi^2 = 5.64$, $P = 0.02$).

4. Discussion

The DRSTP is a tropical developing country; climatic and living conditions favour many parasites (Pampiglione et al., 1987), including *T. gondii*. However, systemic studies regarding the prevalence of *T. gondii* infection in the DRSTP are unknown to date.

The present study is the first report indicating that the overall seroprevalence (21.49%) of *T. gondii* infection in pre-schoolchildren <5 years of age in the DRSTP was higher than that of pre-schoolchildren aged 1–5 years in Somalia in Eastern Africa (8.8%) (Ahmed et al., 1988).

It is generally known that *T. gondii* prevalence is not significantly different between males and females (Montoya and Liesenfeld, 2004) and this was confirmed in the present study. High LA titres of anti-*Toxoplasma* antibodies might be regarded as predictive of the occurrence of acute toxoplasmosis (Kook et al., 1999). In the present study, high *Toxoplasma* LA titres ($\geq 1:1024$) were found in most of the seropositive boys but in fewer seropositive girls. This might be explained by boys having more frequent contact with soil, thus leading to increased opportunities for newly acquired *T. gondii* infection through constant exposure to *T. gondii* oocysts.

It is acknowledged that seroprevalence increases with age, as shown in the data from various countries (Fan et al., 2001, 2002, 2003; Montoya and Liesenfeld, 2004). In this study, a higher seroprevalence and higher LA titres were observed among children aged ≥ 4 years than younger children aged <2 years. The reason for the rise in quantitative titres with age is not clear. A hypothesis would be that the increase is a reflection of increasing 'exposure years' as the children get older (Taylor et al., 1997). Moreover, it was postulated that *Toxoplasma* antibodies with low LA titres in younger children <2 years of age might be from maternal antibodies. It was suggested that protective maternal antibodies might persist until 12 months of age, but that titres declined increasingly with age (Montoya and Liesenfeld, 2004). However, further studies should be performed to differentiate maternal from fetal antibodies by advanced immunological tests, i.e. western blot (Nielsen et al., 2005). On the other hand, high LA titres in older children seemed to indicate acute infection, as it was postulated that they had more chance than younger children of exposure to *T. gondii* infection owing to their greater activity. Nevertheless, in this study it is regrettable that maternal sera were not examined and thus whether seropositive cases were infected congenitally was unclear.

It has been established that *Toxoplasma* infection is prevalent worldwide in humans and animals but the frequency of infection varies from one country to another. This variation is presumably due to the presence or absence of

cats, climatic factors and consumption of raw or improperly cooked meat (Montoya and Liesenfeld, 2004). However, meat consumption for the people of the DRSTP is not easy and is infrequent owing to economic problems. In this study, no attempt was made to determine the source of infection, but considering the abundance of cats (both domestic and stray) and the suitable climatic conditions for sporulation of *Toxoplasma* oocysts in tropical regions (Frenkel et al., 1975), it seemed likely that exposure to cat's faeces or contact with soil contaminated by *Toxoplasma* oocysts was one of the most important factors associated with *Toxoplasma* infection in the early years of life owing to children living and playing very close to the soil, even before they could walk. Such a route of transmission would explain the similar incidences of seropositivity between boys and girls in the DRSTP.

The high prevalence of infection documented in this study indicates a need to enforce methods of control and preventative measures against *Toxoplasma* infection in pre-schoolchildren in the DRSTP. These measures include public education regarding the sources of infection and means of reducing it, such as prevention of soil contamination by cat faeces in areas where children play.

Conflicts of interest statement

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

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