Research Articles

Toxoplasma gondii seroprevalence among two selected groups of pregnant women

SDLP Subasinghe[1], ND Karunaweera[1], A Kaluarachchi[2], CA Abayaweera[1], MH Gunatilake[1], J Ranawaka[2], DMCS Jayasundara[3], GSA Gunawardena[1]

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Abstract

Toxoplasma gondii is an intracellular parasite able to cross the placental barrier and known to infect foetal tissues leading to abortions and congenital deformities. A case control study comparing the seroprevalence of *T. gondii* in 100 healthy pregnant women within 28 weeks of pregnancy and 100 women having undergone a spontaneous miscarriage in the past 6 months attending the Antenatal and Gynaecology clinics of the Professorial Obstetrics & Gynaecology Unit of the De Soyza Maternity Hospital for Women in Colombo was conducted between April 2009 and 2010. Serum was tested for antibodies against *T. gondii* using *OnSite* Toxo IgG/IgM Rapid Test-Dip Strip®. Personal details and data regarding the known risk factors for the infection were obtained using an interviewer administered questionnaire. The participants were aged between 15 and 46 years (median 29); 38% of women in each group were primigravidae. All participants were sero-negative for anti-*T. gondii* IgM antibodies. However, 22.5% (n=45) of all study subjects were sero-positive for anti-*T. gondii* IgG antibodies, which included 62.2% (n=28) from the healthy group and 37.8% (n=17) from those with a recent past history of a spontaneous miscarriage. The difference in seropositivity for *Toxoplasma gondii* between the two selected groups was not statistically significant (X^2=3.47; p=0.063). There were no significant associations between sero-positivity and known risk factors either (p>0.05). Although the study did not reveal any evidence for association between exposure to *Toxoplasma gondii* infection and spontaneous miscarriage, the presence of more than 75% non-immune women of child bearing age is a cause for concern considering the potential risks posed by this parasite, emphasizing the importance of an organized educational programme targeting this high risk group to prevent infection during pregnancy.

[1] Department of Parasitology, Faculty of Medicine, University of Colombo, Sri Lanka
[2] Department of Gynaecology and Obstetrics, Faculty of Medicine, University of Colombo, Sri Lanka
[3] Obstetrics and Gynaecology Professorial Unit, De Soyza Maternity Hospital for Women, Colombo, Sri Lanka

Address for correspondence : Sharmini Gunawardena, Department of Parasitology, Faculty of Medicine, University of Colombo, Sri Lanka email: *sharminigunawardena@hotmail.com*

Introduction

Toxoplasmosis is caused by an obligate intracellular tissue protozoan parasite *Toxoplasma gondii*, which is able to infect humans as well as other warm blooded domestic and wild animals. The infection has a worldwide distribution with approximately one-third of the world population estimated to be exposed to this parasite.[1] There are three forms of *T. gondii* during its life cycle[2]: (i) oocysts are the product of sexual reproduction, which occur in the intestinal epithelium of a cat (the definitive host) that has recently ingested tissue cysts, usually in uncooked meat (ii) tachyzoites are the rapidly dividing products of asexual reproduction, which occur following invasion of the host intestinal wall by either sporozoites (from oocysts) or bradyzoites (from tissue cysts) and (iii) bradyzoites contained within tissue cysts.

Oocysts are passed in the feces of cats and become infectious within 21 days of being shed.[3] Tachyzoites survive and multiply only in an intracellular location while tissue cysts containing few or many bradyzoites occur in the tissues of infected animals within a week of infection.[4] Ingestion of tissue cysts in infected meat and oocysts from soil, food, or water contaminated with cat feces are the two major routes of transmission.[5,6] Rarely, blood transfusions containing infective leucocytes or infected organ transplantations have been implicated.[1,7]

In immunocompetent subjects, 90% of *T. gondii* infections are asymptomatic.[8] Symptomatic infections usually cause low grade fever, malaise, headache and cervical lymphadenopathy. Severe manifestations such as encephalitis, myocarditis, hepatitis and pneumonia are rare but can complicate acute toxoplasmosis[9] and may even lead to death in immunocompromised patients.[1] The importance of this parasite is mainly in pregnancy as it can cross the placental barrier to infect the foetal tissues and thereby cause congenital deformities. If acquired during pregnancy as a primary infection, the parasite can cross the placenta, leading to spontaneous miscarriage, death of the foetus in utero or severe congenital defects such as hydrocephaly, mental retardation or chorioretinitis.[10,11,12,13] A study in Egypt showed that the seroprevalence of antibodies to *Toxoplasma* was higher in women with spontaneous miscarriage compared to a control group.[14]

Active primary infection in the mother can be vertically transmitted to the foetus during any trimester of pregnancy with the risk of severe congenital infection being maximal during the first.[1] Transmission of the parasite to the foetus is dependent on the time of pregnancy that maternal infection is acquired, with a mean transmission rate between 29.0 and 35.0%.[15,16] Although transmission rates are highest during late pregnancy, congenital infections are mild, while primary maternal infection acquired during early pregnancy would lead to significant morbidity and mortality in developing feotuses.[17,1] The risk to the foetus during subsequent pregnancies is minimal.

The clinical spectrum of congenital infection by *T. gondii* ranges from apparent developmental alterations at birth such as microcephaly, intrauterine growth retardation and hydrocephalus which carry high perinatal morbidity and mortality, to subclinical infection, with a risk of developing retinochoroiditis and/or later complications.[18,19] Several studies have suggested *T. gondii* in the causation of spontaneous miscarriage.[20,21,22] The incidence of congenital toxoplasmosis is variable ranging from one in 1000 to one in 12000 births in various countries.[18]

Early diagnosis of toxoplasmosis as well as suitable anti-parasitic treatment of pregnant women, have shown to be effective in reducing severity, although they do not greatly alter the possibility of foetal infection.[16]

The seroprevalence within Asian countries can be classified into 3 groups: high (> 40%), intermediate (10-40%), and low (<10%), with Sri Lanka in an intermediate category having a seroprevalence between 10.0% and 40.0%.[23] Previous studies in Sri Lanka have investigated the prevalence of antibodies to *T. gondii* in adults and neonates,[24,25] healthy adult populations[26] and in pregnant women.[27,28] The seroprevalence rates among pregnant women were 27.5% [27] and 51.4%[28] with a higher seroprevalence (34.0% and 58.2% respectively) among pregnant women having a previous history of spontaneous miscarriage. According to Pappas et al.,[29] the global status of *T gondii* seroprevalence varied between regions and is a measure of the accumulated exposure to *T. gondii* in a particular social setting as well as being an indicator of the relative protection for a woman in this population against primary infection during pregnancy.

Acquisition of toxoplasmosis is prevented mainly by avoiding risk factors known to transmit the infection. Effective prevention of congenital toxoplasmosis depends mainly on avoidance of infection during pregnancy. Uncertainty about how most women acquire infection results in advice to avoid numerous risk factors, making compliance difficult.[30] Therefore, identification of significant associations between known risk factors and seropositivity to *T. gondii* among vulnerable groups would indeed be helpful in adopting appropriate prevention and control methods as suited for each group.

The present study was undertaken primarily to determine the prevalence of *T gondii* antibodies along with its association with known risk factors among healthy pregnant women who visited the antenatal clinic of the Professorial Obstetrics Unit and women who had a history of a miscarriage within the past six months and presented to the clinic or were admitted to the Professorial Gynaecology Unit of the De Soyza Hospital for Women in Colombo.

Materials and Methods

Study design, setting and population

This was a prospective case control study conducted between April 2009 and April 2010 at the Professorial Obstetrics and Gynaecology Unit of the De Soyza Maternity Hospital for Women in Colombo. The study participants were women who attended the Antenatal and Gynaecology clinics of the Professorial Unit. A total of 200 women were recruited for the study assuming the prevalence of infection of the population as being 50% with an allowable error of 20%. The control group consisted of 100 healthy pregnant women within 28 weeks (first 2 trimesters) of pregnancy having no medical complications. The test group was 100 women with a history of a spontaneous miscarriage within 28 weeks of pregnancy and who presented within six months of the event. Women with other medical conditions which could cause miscarriage were excluded from the study to minimize possible confounders. Two hundred consecutive women fitting the definitions were recruited for the study after obtaining informed written consent.

Data collection

Personal details of the participants such as age, parity, period of gestation as well as information regarding association with known risk factors for infection such as contact with cats (cat at home

or in the immediate neighbourhood), the consumption of undercooked meat, past history of blood transfusions or organ transplantation were collected using an interviewer-administered questionnaire. Two milliliters of venous blood was collected from each participant into individual blood collecting tubes and brought to the Department of Parasitology, Faculty of Medicine, University of Colombo, where serum was separated by centrifugation and stored at -20^0C until tested. The serum was tested for the presence of *Toxoplasma gondii* specific antibodies IgM and IgG using the *OnSite* Toxo IgG/IgM Rapid Test-Dip Strip®, a lateral flow chromatographic immunoassay, according to the manufacturer's guidelines. A limitation of this test kit is that the manufacturer does not divulge the level of detection of the specific antibody.

Data analysis

Data was analyzed using the SPSS 15.0 for Windows software package (SPSS Inc., Illinois, Chicago). The Chi-square test was used to determine statistical significance of *T. gondii* seroprevalence between the test and control groups. The association between *T. gondii* seropositivity and risk factors was determined by logistic regression analysis.

Ethical clearance

Ethical clearance for the study was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Colombo (EC-09-018).

Results

General characteristics

The ages of the participants ranged between 15 and 46 years with mean ages being 28.5 and 30.3 years in the control and test groups respectively. Thirty eight percent of women in each group were primigravidae. Eleven and twenty three percent of women of the control and test groups respectively, disclosed a past history of miscarriage which had no significant bearing on *T. gondii* seropositivity (x^2=0.02; p=0.888).

Seroprevalence to *T. gondii*

Table 1 shows the *T gondii* seropositivity among the two selected groups. None of the participants were positive for *T. gondii* specific IgM antibodies. Difference in seropositivity between the control and test groups was not statistically significant (x^2=3.47; p=0.063). Figure 1 shows the distribution of seropositives according to age.

	Number of IgG positives (%)	Number of IgG negatives
Control group	28	72
Test group	17	83
Total	45 (22.5)	155 (77.5)

(x^2=3.47; p=0.063)

Table 1. Number of seropositives for *T. gondii* IgG antibodies among the two groups of pregnant women

Spontaneous miscarriages (currently or in the past) had occurred among only 46.7% (n=21) of women who were seropositive for *T. gondii* specific IgG antibodies (n=45) while miscarriages

had occurred among 58.4% (n=90) of women who were seronegative and this difference was not statistically significant (x^2=1.957; p=0.162). A significant majority of seropositive women (63.4%, n=26) were in their 2nd trimester of pregnancy when compared to the seronegatives (45.6%, n=62; x^2=4.0; p=0.05).

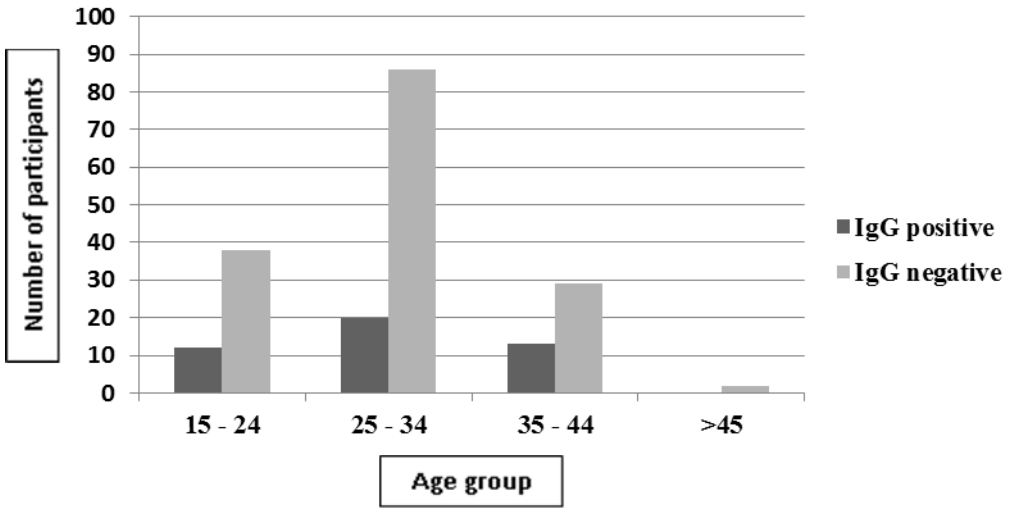

Figure 1 Distribution of *T. gondii* seropositivity according to age.

Association between seroprevalence to *T. gondii* and known risk factors

The associations between known risk factors for *T gondii* infection and seropositivity were analyzed and shown in Table 2. However, none of the known risk factors were significantly associated with *T gondii* specific IgG seropositivity.

Risk factor	Chi square value	*p value*
Age	3.168	0.366
Presence of a cat at home	0.028	0.867
Presence of a cat in the neighbourhood	0.307	0.579
Consumption of raw / undercooked meat	0.025	0.874
Past history of blood transfusion(s)	0.005	0.942

Table 2. The associations between known risk factors for transmission of Toxoplasmosis and *T. gondii* specific IgG seropositivity

Discussion

Seroprevalence of *T gondii* is a measure of the accumulated exposure during a person's lifetime in a particular social setting and therefore the relative protection for a woman in this population against primary infection during pregnancy.[29] In areas with low seroprevalence in the population, the potential for a woman to be infected is consequently low, but if she is infected during pregnancy it will most likely be her primary infection, at risk for abortion or congenital toxoplasmosis. In areas with a high seroprevalence, the chances of a woman acquiring a primary infection during pregnancy are low because she is most likely to have been exposed to *T. gondii* previously. However, if she has not been exposed to the parasite until child-bearing age, the chances of her having a primary infection during pregnancy are high.[29]

The seroprevalence of *T gondii* varies greatly within as well as between regions. The seroprevalence in women of child-bearing age in United States of America, Brazil, Argentina and Colombia was 11.0%, 7.3–77.5%, 48.7–53.4%, and 47.0-63.5% respectively while in Europe it varied between 8.2% (in Switzerland) and 63.2% (in Western Pomerania, Germany). In Asia and Oceania, the seroprevalence ranged from 0.8% (Suwon region, South Korea) to 63.9% (Babol, Iran) and in Africa it was between 25.3% (Burkina Faso) and 75.2% (Sao Tome and Principe). [29]

The prevalence of *T gondii* specific IgG seropositivity of 22.5% (n=45) among the women in the present study is comparable to a previous local study done in 1994[27] which showed a prevalence of 27.5% in a group of 291 pregnant women using an indirect immunofluorescent antibody test (IFAT) for *T. gondii* specific antibodies (at titres of 1:16 or higher). A study done by Ekanayake and Kurukulasuriya (1995)[28] revealed a higher prevalence of 51.4% (n=215) in a group of healthy pregnant women and 58.2% (n=78) among women with threatened and habitual abortions using IFAT and indirect haemagglutination tests (IHT) at titres of 1:16 or higher. A recent study done in Kandy and Kegalle districts by Kurukulasuriya et al. (2009)[26] among apparently healthy populations using the direct Modified Agglutination Test (MAT) revealed an overall prevalence of 27.9% with a slightly higher prevalence among females (31.0%).

The slightly lower seroprevalence in the current study population may be due to the present day urban women being more educated, with better sanitation and hygienic practices and less commonly involved in farming unlike in the past. None of the study subjects were found to be positive for anti-*Toxoplasma* IgM antibodies. Generally IgM antibodies are detected within the first 2 weeks of infection and reduce to negligible levels within 6 months after exposure. However, in toxoplasmosis, IgM titres can remain elevated up to a year or even more. Thus, the mere presence of IgM antibodies is not diagnostic of an acute toxoplasmosis infection. However, a negative IgM antibody test rules out recently acquired infection unless the serum is tested too early after exposure so that antibodies have not as yet developed. A single positive IgG antibody test indicates chronic infection, which might have been acquired before conception, thus posing no risk to the foetus.[15] None of the women tested in our study group were positive for acute infection.

The difference in anti-*Toxoplasma* IgG seropositivity observed between the test and the control groups in our study was not statistically significant. This result tallies with the findings of Samarasinghe[27] in 1994 in which, the differences observed between four selected groups of

women (normal primigravidae, normal multigravidae, women with a single miscarriage only and women with bad obstetric history) was not statistically significant although the group of women with a single spontaneous miscarriage had a higher seropositivity rate (34%) than the normal pregnant women (26.1% - 26.6%) or those with a bad obstetric history (24.4%). Similarly, Ekanayake and Kurukulasuriya[28] too did not find any significant association between women having undergone spontaneous miscarriage and seropositivity to toxoplasmosis. The women who gave a past history of miscarriage in both the control (36.4% Vs. 27.3%) as well as test (17.4% Vs. 16.9%) groups did have a higher seroprevalence to *T. gondii*, although the differences were not statistically significant. This is in keeping with the accepted knowledge about the role of toxoplasmosis in causing sporadic miscarriages.[27] Surprisingly, out of all seropositive women only 46.7% had a history of spontaneous miscarriage at any point in time (in the past and / or at present) which was comparable to the seronegative women ($p>0.05$).

A higher prevalence of *T. gondii* infection has typically been associated with warm and humid environments, contaminated water supplies, poor cooking habits, lack of hygiene, and contact with cats.[31] Yet none of the risk factors considered in the present study showed a significant association with *T. gondii* seropositivity. 44.4% of seropositive women belonged to the age range between 25 to 34 years which is the child bearing age of most women and 46.7% of seropositive women had contact with cats either at home or in the immediate neighbourhood. Cats are popular pets among Sri Lankans and the environmental conditions in the island throughout the year are ideal for the sporulation of oocysts. Kulasiri et al (1965)[32] revealed that 23.7% of cats examined by them were infected with *T. gondii*. Consumption of undercooked meat was rare in the study group in keeping with the culture of Sri Lankans who prefer to cook their meat products well.

In a study conducted in Ottawa by Carter *et al.* (1989),[33] a ten-minute audio-visual educational programme, together with a handout focusing on the current knowledge regarding congenital *T. gondii* infection, effects of foetal infection, and prevention of infection was effective in modifying the behaviour of pregnant women with regard to cats, food preparation and personal hygiene. Although the current study does not support a role for exposure to toxoplasmosis in increasing the risk of premature termination of pregnancy, the presence of 77.5% non-immune women of child bearing age in the population is a cause for concern considering the potential risks posed by this parasite. Therefore, it is recommended that women of child bearing age, including girls attending secondary school be educated regarding risk factors contributing to toxoplasmosis as well as the importance of taking adequate preventive measures, especially during pregnancy.

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