Toxoplasmosis awareness, seroprevalence and risk behavior among pregnant women in the Gampaha district, Sri Lanka

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Background: Primary gestational toxoplasmosis can be transmitted to the fetus with deleterious effects on the pregnancy. There is very little information regarding gestational toxoplasmosis in Sri Lanka. This survey was done to determine the prevalence and awareness of toxoplasmosis and to identify risk factors of infection among pregnant women in the Gampaha district, Sri Lanka.

Methods: Women attending obstetric clinics at the Colombo North Teaching Hospital in 2014 were tested for *Toxoplasma gondii* (*T. gondii*) specific Immunoglobulins G (IgG) and M (IgM) subtypes using the OnSite Toxo IgG/IgM Rapid Test-Dip Strip®. Disease awareness and risk behaviors of the participants were investigated.

Results: Of the 293 participants (mean age 27 years, SD $\pm$ 5.92), 38% were primigravidae with a mean gestational age of 16.2 weeks (SD 7). The prevalence of anti-*T. gondii* IgG and IgM antibodies was 12.3% (n = 36) and zero, respectively. Unadjusted and adjusted odds ratios were calculated to determine risk factors of infection (cat-ownership, handling cats, consumption of meat, commercial meals and unwashed raw vegetables and fruits, handling soil and not washing hands after handling soil). On bivariate analysis, eating commercially prepared meals weekly or more was associated with toxoplasma seroprevalence with marginal statistical significance. On multivariate analysis, none of the considered risk factors were significant. Toxoplasma awareness was 4.4% (n = 13); health personnel (46.2%, n = 6) and media (53.8%, n = 7) being sources of information.

Conclusions: Health education programs to increase awareness of toxoplasmosis is recommended at antenatal clinics.

Keywords: Awareness, Pregnancy, Seroprevalence, Sri Lanka, *Toxoplasma gondii*, Risk factors

Background

Toxoplasmosis is caused by infection with the zoonotic protozoan parasite, *Toxoplasma gondii*. It infects a wide range of hosts and is distributed worldwide. Because of the current high rates of infection among the human population (estimated as 30–50%) it is considered a global health hazard.¹ Toxoplasmosis is characterized by a wide range of clinical syndromes. The majority of infections are typically asymptomatic or cause a mild self-limiting ‘flu’ like illness. Primary gestational infections can be transmitted to the fetus causing congenital toxoplasmosis which may result in miscarriages, death in utero, neonatal growth retardation, neurological or ophthalmic disease.^{2–4} The estimated global burden of congenital toxoplasmosis was 19,000 new cases each year resulting in the loss of approximately 1.2 million DALYs.⁵

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Cats and other felines are the definitive hosts of *T. gondii* and thus the only source of environmentally resistant oocysts in nature. Major routes of infection for humans are: ingestion of oocysts through close contact with infected cats or cat excreta, ingestion of water, food or soil contaminated with oocysts; ingestion of tissue cysts by handling or consumption of inadequately cooked infected meat of intermediate hosts, transplantation of infected organs and congenital infection.⁶

Antenatal screening for *T. gondii* infection based on antitoxoplasma-specific IgG and IgM detection is the mainstay in monitoring the risk of congenital toxoplasmosis. Maternal-fetal intervention can be achieved through drugs such as spiramycin which prevent congenital toxoplasmosis by more than 60%.⁷ There is no antenatal screening program for pregnant women in Sri Lanka.

Rates of exposure to the organism vary greatly according to the geographical location, socioeconomic status, cultural

and religious beliefs of a population.⁸ Hence, there is a need to ascertain the prevalence in different settings. Evidence of zoonotic infection among cats and livestock animals of Sri Lanka indicates the existence of the parasite and hence the potential for human infection.^{9–11} Recent trends in foods and lifestyle with fast foods replacing traditional cooking and popularity of companion animals, (especially cats, as they require minimal care) and the abundance of stray cats, could increase the likelihood of exposure to toxoplasmosis. Therefore, vigilance is required with regard to toxoplasmosis. Seroprevalence among females and neonates have been studied in and around Colombo in the past.^{12–15} However, no studies have investigated the awareness of toxoplasmosis and related preventive behavior patterns among pregnant women in Sri Lanka.

Prevention of congenital toxoplasmosis depends mainly on avoidance of risk factors during pregnancy. Uncertainty about how most women acquire infection results in advice to avoid numerous risk factors which makes compliance difficult. 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 objectives of this study were to identify the toxoplasma seroprevalence and disease awareness among a semi-urban population of pregnant females, residing in the Gampaha district and if possible to identify risk factors and routes of infection that play an important role in the transmission of toxoplasmosis in the area.

Methods

A cross-sectional survey was done at the obstetric clinics of Colombo North Teaching Hospital (CNTH) in Ragama from February to June 2014. Ragama is a small town situated in the district of Gampaha, in the Western Province of Sri Lanka. Its geographical coordinates are 7.0308°N, 79.9167°E and it lies at an elevation of 8 m above sea level. The hot, humid, climate that prevails throughout the year is suitable for the sustenance and sporulation of *T. gondii* oocysts in soil. Rice eaten with curries (vegetable, fish, chicken, pork and beef) and salads prepared from raw leafy vegetables is the staple diet in the country. The CNTH, with a bed strength of over 1000, is the second largest public hospital in Sri Lanka, and is the primary teaching hospital of the Medical Faculty of the University of Kelaniya.

The sample size was calculated to estimate the prevalence of toxoplasma antibodies in this population. For the calculation, prevalence was assumed to be 25% based on previous research.^{14,15} Considering a confidence interval of 95% and the acceptable difference to be 5%, the minimum required sample size was 289. We obtained a sample size of 291 by recruiting all consenting pregnant woman at their first visit to the antenatal clinic at the CNTH over a three-month period.

The study was approved by the Ethical Review Committee of the Faculty of Medicine University of Kelaniya. Informed written consent was obtained from the participating couples at the first booking visit. Unmarried subjects which included a few minors ($n = 3$), consulted the guardian (mother/mothers-in law) who accompanied them to the antenatal clinic prior to signing the consent forms. The participants were informed that enrolment to the study was purely voluntary and non-participation would not in any way influence the services provided by the clinic.

A pretested questionnaire was administered by trained research assistants to gather information. The questions elicited information on four major areas: (a) socio-demographic information of the participants such as age, ethnicity, level of education, employment status, marital status, area of residence; (b) details of prior adverse birth outcomes, from multigravida i.e. miscarriages, still births, children with delayed milestones; (c) disease-related risk factors such as cat ownership, personal hygiene (frequency of hand washing), kitchen hygiene (frequency of washing utensils, cutting board and knife after preparation of meat), consumption of meat, cooking of meat, frequency of consuming unwashed raw vegetables and fruits, frequency of having non-homemade meals and exposure to soil; and finally, (d) the awareness of toxoplasmosis and sources of information. After completing the questionnaire, each subject was educated on importance and prophylactic measures against toxoplasmosis using an information sheet.

Two milliliters of blood was collected aseptically from each participant into sterile containers and transported to the laboratory of the Department of Parasitology, Faculty of Medicine, University of Kelaniya. At the laboratory, the serum was separated by centrifugation and tested on the day of receipt for *T. gondii*-specific antibodies using a commercial kit (*OnSite Toxo IgG/IgM Rapid Test-Dip Strip*® CTK Biotech. Inc. USA) according to the manufacturer's instructions. The participants were made aware of the results of the hematological investigations and its interpretation.

The data from the questionnaires and laboratory results was stored in an EpiInfo database and analyzed using the statistical software package SPSS version 17. Bivariate and multivariate analyses were done for analytic assessment of considered risk factors.

Results

A total of 293 pregnant females were surveyed. The socio-demographic and past obstetric data of the population is detailed in Table 1. The mean age of the study population was 27 (SD, ± 5.92) years. The majority of the subjects had completed their basic education, with over 70% successfully completing the first national school examination (GCE Ordinary Level Examination). However, only a few of them were employed (10 and 15% on permanent and temporary basis, respectively).

Table 1 Association between toxoplasma seroprevalence and socio-demographic variables of the population

Variables	Toxoplasma IgG		Bivariate analysis	Multivariate analysis
	Positive number (%)	Negative number (%)	OR (95% CI)	OR (95% CI)
<i>Age group (n = 287)</i>				
16–29 years	27 (14.1)	165 (85.9)	1	1
30–40 years	9 (9.5)	86 (90.5)	0.640 (0.288–1.421)	0.619 (0.269–1.424)
<i>Educational level (n = 292)</i>				
Primary education or less	2 (11.1)	16 (88.9)	1	1
Grade 8 passed	12 (18.5)	53 (81.5)	1.811 (0.366–8.953)	1.263 (0.240–6.658)
O/L passed	19 (12.3)	136 (87.7)	1.118 (0.238–5.247)	0.697 (0.138–3.521)
A/L passed	3 (5.6)	51 (94.4)	0.471 (0.072–3.069)	0.220 (0.029–1.658)
<i>Occupation (n = 291)</i>				
Permanent	6 (20.7)	23 (79.3)	1	1
Casual	4 (9.3)	39 (90.7)	0.393 (0.100–1.541)	0.282 (0.066–1.198)
Housewife	26 (11.9)	193 (88.1)	0.516 (0.192–1.386)	0.366 (0.124–1.081)
<i>Marital status (n = 290)</i>				
Unmarried	4 (22.2)	14 (77.8)	1	1
Married	32 (11.8)	240 (88.2)	0.467 (0.145–1.505)	0.667 (0.186–2.393)
<i>Ethnicity (n = 292)</i>				
Sinhala	33 (13.5)	211 (86.5)	1	1
Non-Sinhala	3 (6.3)	45 (93.8)	0.426 (0.125–1.451)	0.352 (0.099–1.255)

Thirty-nine percent ($n = 115$) of the study population were primigravidae. The mean gestational age of the population was 16 weeks ($SD \pm 7$). Among the multigravidae, ($n = 178$), 45% reported adverse birth events (miscarriages, neonatal deaths, delayed milestones and congenital anomalies in 29.2, 3.9, 8.1 and 3.9%, respectively) in their past obstetric history.

Toxoplasma-specific IgG antibodies indicating past exposure was detected in 12.2% ($n = 36$) of the study population. IgG seroprevalence was higher among the younger age group (16–29 years), compared to the older participants (30–40 years), but the difference was not statistically significant (14.1 and 9.5%, respectively, $p > 0.05$). None of the participants were positive for toxoplasma-specific IgM antibodies during the three-month study period. None of the socio-demographic factors or adverse obstetric outcomes studied showed a significant association with toxoplasma seropositivity.

Only 4.4% ($n = 13$) of the pregnant females surveyed were aware of toxoplasmosis and public health midwives (23%, $n = 3$), doctors (23%, $n = 3$), newspapers (23%, $n = 3$) and television (30.7%, $n = 4$) were the sources of information.

The risk factors related to toxoplasmosis among the study participants are given in Table 2. Although toxoplasma seroprevalence was higher among those with positive risk factors, a significant association was not seen with any of the considered risk factors on multivariate analysis. In the bivariate analysis, eating commercially prepared food weekly or more was associated with toxoplasma seroprevalence with marginal statistical significance.

Discussion

Toxoplasmosis is a global infection with wide variation in prevalence within and between regions. Therefore, it is a major concern in both developed and developing

countries. In the American continent, the prevalence of infection was high in Latin American countries such as Brazil, (68.37%) and Mexico (6.1–27.9%) compared to the USA (17.5–29.2%).^{16–18} In Europe, the prevalence was relatively high in Poland (41.3%) and France (43.8%) compared to Portugal (24.4%), the United Kingdom (17.3%) and Denmark (2.4%).^{19–23} The highest seroprevalence rates were reported from the African continent (Ghana, Ethiopia and Congo 92.5, 81.4 and 84.7%, respectively).^{24–26} Rates among Asian countries ranged from as low as 4, 7.7 and 10.3% in South Korea, Taiwan (Taipei) and Japan, respectively to moderate values of 17, 22.4 and 38.5% in Pakistan, India and Bangladesh, respectively.^{8,27–31}

The toxoplasma seroprevalence of 12.2% derived in the present study was lower than the rates reported in the recent past for females of the reproductive age group residing in Colombo, (22.5 and 27.5% in 2011 and 2005, respectively).^{11,14} The variation of seroprevalence within a country has been previously documented.^{8,18} The significant difference in prevalence between the regions was attributed to differences in socioeconomic, cultural (cat ownership, personal hygienic practices and feeding habits) and climatic factors.^{8,18} The low rate of exposure derived in the present study could be partly attributed to the paucity of risk factors among our study participants. Many households did not own cats (77%, $n = 225$) and contact with cats was minimal 6% ($n = 18$). Even among those reported to be handling cats, the rate of exposure did not differ largely from those not handling cats (16 and 12%, respectively) indicating that this was not a major route of infection. The findings are consistent with studies done in Palestine, Turkey, Nigeria and India.^{8,32–34} Nevertheless studies from Taiwan and West Africa have reported significant associations with *T. gondii* seroprevalence and contact with cats.^{35,36} However, the risk of contracting toxoplasmosis is related to the way the cat's litter is handled, rather

Table 2 Association of toxoplasma seroprevalence with risk behaviors of the population

Variables	Toxoplasma IgG		Bivariate analysis	Multivariate analysis
	Positive Number (%)	Negative Number (%)	OR (95% CI)	OR (95% CI)
<i>Current cat ownership (n = 293)</i>				
No	25 (11.1)	200 (88.9)	1	1
Yes	11 (16.2)	57 (83.8)	1.544 (0.716–3.327)	1.374 (0.407–4.641)
<i>Handling cats (n = 292)</i>				
No	33 (12.0)	242 (88.0)	1	1
Yes	3 (16.5)	15 (83.3)	1.467 (0.403–5.338)	0.000
<i>Consumption of meat (n = 293)</i>				
No	4 (9.8)	37 (90.2)	1	1
Yes	32 (12.7)	220 (87.3)	1.345 (0.450–4.027)	0.559 (0.136–2.292)
<i>Consumption of unwashed raw vegetables and green leaves (n = 255)</i>				
No	28 (12.3)	200 (87.7)	1	1
Yes	3 (11.1)	24 (88.9)	0.893 (0.252–3.159)	0.489 (0.058–4.098)
<i>Eating commercially prepared meals (n = 288)</i>				
Less than once a week	17 (9.5)	162 (90.5)	1	1
Weekly or more	19 (17.4)	90 (82.6)	2.012 (0.996–4.064)*	1.578 (0.616–4.039)
<i>Gardening (n = 291)</i>				
No	11 (9.4)	106 (90.6)	1	1
Yes	25 (14.4)	149 (85.6)	1.617 (0.763–3.428)	1.131 (0.198–6.456)
<i>Washing hands after handling soil (n=195)</i>				
Yes	26 (14.4)	154 (85.6)	1	1
No	2 (13.3)	13 (86.7)	0.911 (0.194–4.274)	0.868 (0.084–8.978)
<i>Washing cutting utensils after handling meat (n=251)</i>				
Yes	27 (11.9)	199 (88.1)	1	1
No	3 (12.0)	22 (88.0)	1.005 (0.282–3.584)	1.053 (0.206–5.383)

*Marginally significant association.

than mere contact with cats. This is because oocysts, which require a few days of development in the environment to be infective, are generally not found on cat's fur but in cat excreta.³⁷ Contact with cat excreta was identified as a significant risk factor for toxoplasma seropositivity in Accra, Ghana, where the seroprevalence among pregnant females was reportedly high (92.5%).²⁴

Subasinghe et al. in 2011, reported that 46% of the women who were seropositive for *T. gondii* in Colombo had contact with cats either at home or in the neighborhood.¹⁵ The authors were unable to show a significant association between cats and seropositivity but the data suggested the presence of a more abundant population of cats and hence the likelihood of more numerous oocysts in and around Colombo, compared to Ragama (contact with cats among seropositives was only 16% in Ragama) which could be partly responsible for less exposure.

Soil is a major source of infective oocysts as cat excreta is buried in soil.³⁸ Contact with soil is universal and difficult to avoid, therefore establishing a link with *T. gondii* seropositivity is difficult. In the current survey, only 59% reported gardening as a pastime but contact with soil was reported by all participants. There was no association between gardening and exposure to infection in this study. Contact with soil and the presence of a cat in the area was shown to be significantly associated with *T. gondii* seropositivity in Central Ghana.³⁹

Personal hygiene of the study participants appeared to be of high order. Majority (95%) washed their hands after

risky behavior (handling cats, soil and raw meat) and washed raw fruits and vegetables prior to consumption (78%) thus minimizing the risks. Most of the participants were involved in housekeeping and apparently maintained good kitchen hygiene reducing the chances of infection via contaminated kitchen utensils. Although meat consumption was relatively high, the lengthy cooking process of traditional curries makes this a most unlikely route of infection.

Over half of the population reported consumption of commercially prepared meals from small food outlets. Eating out of home and from places of uncertain hygiene were shown to be significant risk factors of toxoplasmosis among interstate truck drivers in Mexico.⁴⁰ In the present survey, a marginally significant risk of exposure among frequent consumers of commercial meals was evident on bivariate analysis but not on multivariate analysis possibly due to the effect of low sample numbers.

Most studies have reported that age is a major variable of a higher prevalence rate of toxoplasmosis.^{7,8,24,32} The tendency for seroprevalence to increase with age reflects the accumulated exposure to infection during a person's lifetime. However, in this study a slightly higher seroprevalence was observed among the 16–29 age group as compared to older females aged 30 and above. But the difference was not statistically significant. The reason for a decline in seroprevalence with age is not clear. This could be an effect of the small sample size of the older age group.

The risk of congenital toxoplasmosis is with maternal parasitaemia that is seen only when a non-immune mother

is exposed to infection.³ In areas with low seroprevalence, the potential for pregnant women to get infected is consequently low but if she is infected, it will most likely be her primary infection and the baby will be at risk of congenital toxoplasmosis. Therefore, creation of disease awareness is important during pregnancy especially as the majority of the participants were non-immune (88%). The toxoplasma awareness among the study population was almost non-existent (4.4%). Evidence from previous studies underscores the importance of health education on toxoplasmosis. A study in Belgium demonstrated that health education was associated with a 63% reduction in *T. gondii* seroconversion among pregnant women.⁴¹

Therefore establishment of a health education program targeting women of reproductive age is a priority.

Women contemplating pregnancy should be educated about eating meals that are hygienically prepared, not eating undercooked meat, using measures to avoid cross-contamination of cooked foods with raw or undercooked meat and protecting themselves against exposure to cat litter or contaminated soil. The health message could be delivered via multiple approaches such as media (newspapers, magazines and television) public health workers and health personnel at antenatal clinics.

Results of this study cannot be generalized to the whole country as it focused on females attending the CNTH, mostly a semi-urban educated population. Levels of exposure to toxoplasmosis could vary in more rural areas where involvement in agricultural activities and soil contact is greater. Therefore, surveys need to be done on a country-wide basis as regional variation is well documented.

This study had a few limitations. A major limitation of this study was the use of only a single rapid test to detect toxoplasma seropositivity owing to financial constraints. The above factor could be a contributor to the relatively low seroprevalence derived if the screening tool was of low sensitivity. However, the high sensitivity of the kit as given in the kit literature (91.6 and 100% on comparison to a commercial EIA for IgG and IgM antibodies, respectively) suggests accuracy. The assessment of risk factors was based on information acquired by interviewing the participants. Information pertaining to hygienic practices may be misrepresented due to shame and embarrassment but the low infection rates derived reflect the accuracy of the data.

Conclusions

The importance of the current study is that it is one of the few surveys done in Sri Lanka which assessed disease knowledge and risk factors in addition to seroprevalence. Also the study was done in an area that was hitherto not surveyed. Therefore, it provides information on regional variation on exposure to *T. gondii* and specifies the remedial measures important to minimize exposure in this community. Based on the results of this survey and those reported previously the necessity of routine toxoplasma

screening at antenatal clinics needs to be considered in Sri Lanka due to the non-immune status of the majority although the risk of infection appears low. However creation of toxoplasma awareness among females of the reproductive age group is important. Implementation of strict hygienic measures in the food industry by public health authorities is also recommended.

Conflict of interests

The authors declare that they have no conflict of interests

Author's contributions

NC conceived the study; NC and RH designed the study protocol; NR, BS, HS and AK carried out the clinical assessment and analysis and interpretation of data, NC and RH drafted the manuscript; NRDS critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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