Seroprevalence of *Toxoplasma gondii* infection among pregnant women in Cameroon

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Abstract

Toxoplasmosis is caused by an intracellular protozoan, *Toxoplasma gondii*, which has a wide geographical distribution. The congenital form results in a gestational form that can present a temporary parasiteamia that will infect the fetus. For this reason early diagnosis in pregnancy is highly desirable, allowing prompt intervention in cases of infection. The aim of this study was to determine the seroprevalence of *Toxoplasma gondii* antibodies among pregnant women attending the Douala General Hospital. The study was carried out between March and July 2009, whereby 110 pregnant women were tested for IgG and IgM antibodies and information about eating habits and hygienic conditions was collected using a questionnaire. These women's ages ranged from 20-44 years old with an average of 29.9 years; the overall IgG and IgM seroprevalence was 70% and 2.73 % respectively. Seroprevalence was significantly high amongst women who ate raw vegetables (76.39%, P<0.05) and there was a significant trend towards a higher seroprevalence in women who did not have a good source of water (75.58%, P<0.05). This research showed that consumption of raw vegetables and poor quality drinking water are two risk factors associated with *Toxoplasma gondii* infection amongst pregnant women attending the Douala General Hospital in Cameroon.

Introduction

Discovered in 1908, toxoplasmosis is caused by an intracellular protozoan known as *Toxoplasma gondii*. It infects multiple of warm blooded animals including humans, livestock, birds etc, however cats, sheeps and pigs are the definitive hosts to *Toxoplasma gondii*. Toxoplasmosis has a wide geographical distribution. Although *T. gondii* infection in adults is usually asymptomatic or associated with self limited symptoms (e.g. fever, malaise, lymphadenopathy), infection of humans occur either congenitally or by ingestion of foodstuffs contaminated by infected cat faeces, or lamb or pork contaminated with *T. gondii* cysts. Contamination of pregnant women may cause serious health problems if the parasite is transmitted to the foetus to cause congenital toxoplasmosis.[1] The congenital form results in a severe systemic disease because if the mother is infected for the first time during gestation, she can present a temporary parasitemia that will infect the foetus. Many clinical symptoms are seen in congenitally infected children from a mild disease to serious signs such as mental retardation. Early diagnosis during pregnancy is highly desirable allowing prompt intervention in cases of infection through treatment in order to reduce the probability of foetal infection and consequent substantial damage to the foetus. Conventional tests for establishment of a foetus diagnosis of toxoplasmosis include options from serology to PCR. Prevention of human toxoplasmosis is based on care to avoid the infection, understanding the disease and serological exams during gestation. Pregnant women should be tested serologically from 3 months of gestation until one month after childbirth. Toxoplasma infection during pregnancy is widely treated with oral spiramycin to reduce the risk of congenital toxoplasmosis in the infant. Failures of therapy have been observed.[2] Different serological tests often measure different antibodies that possess unique patterns of rise and fall with time after infection. A combination of serological tests is frequently required to establish whether an individual has been more likely infected in the distant past or has been recently infected.[3] The prevalence rate of toxoplasmosis varies according to geographical location, age, gender of the population studied and other factors. In Europe, the prevalence rate ranges from 20% to 85%; in the United States, the prevalence is 12% to 41%,[4] while in Cameroon is 48.5%.[5] Prevalence in other countries can vary from 18% to 65%.[6] The main objective of this study was to determine the seroprevalence and to assess some risk factors associated with *Toxoplasma gondii* amongst pregnant women who consulted at the Department of Gynaecology in the Douala General Hospital, Cameroon.

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Materials and Methods

Materials

The study was carried out at the Douala General Hospital in the period March-July 2009. The study subjects comprised of pregnant women aged 18-45 years old sent to the laboratory of the Douala General Hospital by the Gynaecology Department for *T. gondii* antibodies screening. Douala serves as the economic capital of Cameroon. Its industrial, economic, commercial and educational services have accounted for the dense population found here. The population growth rate is estimated at 9.3%. The development of the town is uneven with a wide gap between the poor and the rich. Most of the poor do not have access to clean water and have resorted to wells, which are not often treated as their only source of water. There are problems of poor housing conditions, overcrowding, low family income coupled with poverty which have helped in the spread of many parasitic infections. This town has many hospitals, which are distributed evenly with 2 considered as reference hospitals; the General Hospital and Laquintinie Hospital, Douala.

Methods

Sampling and sample collection

Data was obtained through the filling of questionnaire. The questionnaire was designed and pretested at the antenatal clinic of the General Hospital annex of Buea in Cameroon. The atecubical vein of the forearms was selected and disinfected with 70% alcohol cotton wool swab. Venous blood was collected into a dry tube, which was labeled with the patients name and code. The sample was

allowed to clot completely before centrifugation. After centrifugation, serum was separated from the clot into tightly closed storage tubes and stored at 2-8°C since tests were performed in batches once every week.

Laboratory methods

The platelia tm Toxo IgG TMB and IgM kits for the qualitative and quantitative detection of anti-T.gondii IgG and IgM in human serum by enzyme immunoassay was then used to analyse each sample.

Principle of Toxo IgG

The principle of this is a solid phase technique referred to as indirect ELISA. The *T. gondii* antigens used to coat the microplates were obtained from tachyzoites ultrasonic enriched with membrane proteins. The conjugate consist of a peroxydase labeled monoclonal antibody specific to human gamma chains. Reading is converted into IU/mL using a standard curve.

Principle of IgM

The test is an enzyme-linked immunosorbent assay (ELISA), the principle is based on the capture of the IgM on the solid phase. Anti-human antibodies are coated on the solid phase (wells of the microplate). A mixture of the *T. gondii* antigens and the monoclonal anti-*T. gondii* antibody labeled with peroxydase is used as the conjugate. The test uses the following steps.

Assay procedure

Generally, after enough samples have been collected, those in the freezer are removed and thawed at room temperature. These sera are then processed as described by the manufacturer (Human INC).

Interpretation of results for IgG

Detection of the lgG anti-*T. gondii* with Platelia Toxo lgG TMB provides the Patients with their immune status: i) titer<61 U/mL, non significant level of anti body – Absence of immunity; ii) 61 U/mL<Titer<91 U/mL, non significant level of antibody, the result of a single serum sample does not constitute sufficient proof for establishing the patient's immune status against *T. gondii*; iii) 91 U/mL<Titer<240 U/mL, significant level of antibody. Long standing immunity or early seroconversion; iv) titer≥240 U/mL, high levels of antibody. Recent seroconversion or persistently high level of immunity.

Data analyses

Data was entered into Microsoft Excel sheets and exported to Epi-Info for analysis using descriptive statistics. Statistical significance was set at 95% confidence interval (CI). At the initial step of the analyses, frequency distributions of each variable were produced and the information arranged according to age groups and risk factors.

Results

A total of 110 samples from pregnant women were analysed. These women's ages ranged from 20-44 years, with an average of 29.9 years. The prevalence of toxoplasmosis was 77 (70%) giving a 95% confidence interval of 61.4-78.6%. Among these women, 77 women were seropositive for IgG antibodies and 3 were seropositive for IgM antibodies giving a prevalence of 70% and 2.73%, respectively. Here, the various parameters analysed are shown on Tables 1 to 7. Percentages were used to express the prevalence rates.

Table 1 shows the seroprevalence of toxoplasmosis amongst pregnant women classified according to age groups. The highest prevalence was found within the age range of 31-35, but this prevalence does not reach statistical significance.

Table 2 shows a higher seroprevalence of toxoplasmosis amongst pregnant women who owned cats. However, this prevalence was not statistically significant (χ^2=0.48; P=0.12).

Table 3 shows a significantly higher seroprevalence amongst pregnant women who eat raw vegetables and a lower prevalence amongst those who do not eat raw vegetables.

Table 4 shows a higher seroprevalence amongst pregnant women who eat meat and a lower prevalence amongst those who do not eat meat but this higher seroprevalence did not reach statistical significance (P=0.09).

Table 5 shows that the seroprevalence of toxoplasmosis is significantly higher amongst

Table 1. Frequency distribution of age groups of toxoplasmosis in pregnant women with respect to age.

	T. gondii IgG		
Age groups	No of positives (%)	No of negatives (%)	Total (%)
20-25	16 (59.30)	11(40.70)	27 (24.50)
26-30	21 (70.00)	9 (30.00)	30 (27.30)
31-35	24 (70.60)	10 (29.40)	34 (30.90)
36-44	16 (84.20)	3(15.80)	19 (17.30)
Total	77 (70.00)	33 (30.00)	110 (100.00)

Table 2. Prevalence of toxoplasmosis amongst pregnant women based on cats' ownership.

	T. gondii IgG		
	No of positives (%)	No of negatives (%)	Total (%)
Yes	45(72.58)	17(27.42)	62 (56.40)
No	32(66.67)	16(33.33)	48 (43.60)
Total	77(70.00)	33(30.00)	110 (100.00)

χ^2=0.48; P=0.12.

Table 3. Prevalence of toxoplasmosis among pregnant women based on raw vegetables consumption.

	T. gondii IgG		
	No of positives (%)	No of negatives (%)	Total (%)
Yes	55(76.40)	17(23.60)	72 (65.50)
No	22(57.90)	16(42.10)	38 (34.50)
Total	77(70.00)	33(30.00)	110 (30.00)

Table 4. Prevalence of toxoplasmosis amongst pregnant women based on meat consumption.

	T. gondii IgG		
	No of positives (%)	No of negatives (%)	Total (%)
Yes	66(73.30)	24 (26.70)	90 (81.80)
No	11(55.00)	9 (45.00)	20 (18.20)
Total	77(70.00)	33 (30.00)	110 (100.00)

those pregnant women whose source of drinking water is from a general network and significantly lower amongst those whose source of water is other than the general network such as bottled water (P=0.03).

A summary of risk factors to toxoplasmosis in pregnancy in the general Hospital DOUALA, Cameroon is presented in Table 7. The study showed the role of age, cat ownership, consumption of raw vegetables, source of potable water, meat consumption, and gestational age as risk factors for toxoplamosis in pregnancy. This table shows the risk factors of toxoplasmosis and their confidence intervals. The lower and upper limits of these confidence intervals are so closed indicating that the results were correctly measured and that toxoplasmosis is a major public health problem among pregnant women in Cameroon.

Table 5. Prevalence of toxoplasmosis amongst pregnant based on the source of drinking water.

	T. gondii IgG		
	No of positives (%)	No of negatives (%)	Total (%)
Other	12(50.00)	12(50.00)	24 (21.80)
General network	65(75.60)	21(24.40)	86 (78.20)
Total	77(70.00)	33(30.00)	110 (100.00)

χ^2=5.63; P=0.03.

Table 6. Prevalence of toxoplasmosis amongst pregnant women based on the gestational age.

	T. gondii IgG		
	No of positives (%)	No of negatives (%)	Total (%)
First trimester	14(87.50)	2(12.50)	16 (14.50)
Second trimester	39(66.10)	20(33.90)	59 (53.60)
Third trimester	24(68.57)	11(31.43)	35 (31.80)
Total	77(70.00)	33(30.00)	110 (100.00)

χ^2=2.7; P>0.05.

Discussion

Toxoplasmosis is an asymptomatic infection that can have severe consequences if pregnant women become infected. It is thus very essential to determine whether the infection is recent. Traditionally, screening for toxoplasmosis has been carried out in France[7] and Austria[8] as a mandatory part of prenatal care. Prenatal screening has also been carried out in pilot projects in countries such as Finland,[9] Sweden[10] and Brazil.[11] This should also be implemented in developing countries looking at the prevalence (70%) obtained in this study. A wide variability in the seroprevalence of *T. gondii* amongst pregnant women has been reported worldwide. A decrease in the prevalence of the infection has been reported in developed countries as in the United States, where several studies have shown a clear decrease in seroprevalence in several geographic localities. A recent experience in the central valley region of Costa Rica revealed a decrease in seroprevalence from more than 70% in the 1980's to 58% in 2003. When this data was compared with those reported in the 1985 survey in the western part of Cameroon (prevalence of 48.5%)[5] an increase in seroprevalence was noted, as compared with this study revealed (70%). The observed seroprevalence in this study did not depend on age but was higher in women aged between 31-35 years old. This is in contrast with the research done in California, which showed a significantly higher prevalence with increasing age.[6] Seroprevalence was found to be associated to the different drinking water source in the current study. The highest prevalence (75.58%) was in people having as source of drinking water the general network supply. The higher seroprevalence in general network water users may be in accordance with a work published by Bowie *et al.*, who indicated the presence of oocysts in chlorinated network water.[12] The general network supply of water in our cities is generally not well treated and whose supply is generally erratic. The lower prevalence in people using other sources of water such as bottle water users might be explained in that the containers are filled soon after the water surfaces. Oocyst forms of *T. gondii* seem to be the major factor in the infection of water resources.[12] Considering the abundance of domestic and stray cats in the country, the consumption of uncooked vegetables and the suitable climatic conditions for sporulation of *T. gondii* oocysts, it seems exposure to cat faeces is the principal route for toxoplasma infection in most parts of the country. Although cats are the primary source of oocysts that cause human and livestock infections,[13] in our study, household cats were not found to be significantly associated with *T. gondii* infections in pregnant women. This could therefore be due to the consumption of raw vegetables as a significant high prevalence amongst those who eat raw vegetables not properly washed. The finding correlates with an earlier report in Trinidad and Tobago.[14] Oocysts are not found on cats' fur and are often buried in soil along with cats' faeces, and soil contact is universal and difficult to avoid. Vegetbles sold in our markets are usually displayed on plain ground, with the possibility of contamination with cats faeces. Exposure to infected meat could be a risk factor as well, but from this study there was no significant prevalence with meat consumption and a similar situation was observed in Colombia.[6] The convention here is to eat meat that has been well cooked. This study

Table 7. Summary of risk factors to toxoplasmosis in pregnancy in the general Hospital Douala, Cameroon.

Risk factors	Prevalence (%)	Confidence interval (%)	
		Lower limit	Upper limit
Age (31-35 years)	31.2	22.5	39.9
Cat ownership	72.6	64.3	80.9
No cat ownership	66.7	57.9	75.5
Raw vegetable consumption	76.4	68.5	84.3
No raw vegetable consumption	57.9	48.7	67.1
Meat consumption	73.3	65.0	81.6
No meat consumption	55.0	45.7	64.3
Potable water from well	50.0	40.7	59.3
Potable water from a tap	75.6	67.6	83.6
Gestational age: 1st trimester	87.5	81.3	93.7
Gestational age: 2nd trimester	66.1	57.2	74.9
Gestational age: 3rd trimester	68.6	59.8	77.3

showed that the 70% of the pregnant women had a latent *T. gondii* infection based on the presence of IgG antibodies while 2.73% of the pregnant women had an acute infection based on the presence of IgM antibodies. Therefore there is a high risk of transplacental vertical transmission to the fetus in women with IgM antibodies. This study also showed that raw vegetable consumption and the use of general network as source of drinking water are risk factors that can be associated with toxoplasmosis in pregnant women attending the Douala General Hospital in Cameroon.

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