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Seroepidemiology of Toxoplasmosis in Pregnant Women Attending the University Teaching Hospital in Yaounde, Cameroon

Abstract

Purpose: The study was carried out to investigate the epidemiology and associated toxoplasmosis predisposing risk factors in Cameroon.

Methods: The survey took place at the Yaounde University Teaching Hospital from May to June 2008. Serum samples were collected from 110 pregnant women attending the ante natal clinic using aseptic techniques after obtaining informed consent. The samples were analysed using toxo-IgG immunocomb and toxo-IgM “capture” ELISA. A structured questionnaire was used to collect information on predisposing risk factors for toxoplasmosis from each patient. Data was analysed on Epi-Info using confidence intervals and chi-square statistic test.

Results: The average age of the women was 27.9 ± 5.8 years and the mean gestational age was 4.1 ± 0.2 months. The overall IgG seroprevalence was 65.5% {95% CI: 53.7-71.7%} and that of IgG and IgM co-infection was 2.7%. The seroprevalence was 75% in the first trimester, 60.6% in the second trimester and 50% in the third trimester. No statistically significant relationships were established between anti-toxoplasma IgG and IgM antibodies and abortion history, meat consumption, potable water sources, cat ownership and age.

Conclusion: The prevalence of IgG antibodies to *Toxoplasma gondii* is high and the first trimester in pregnancy carries the highest risk. All pregnant women should be screened for toxoplasmosis and educated on predisposing risk factors during antenatal visits.

Keywords: Toxoplasma gondii, Pregnancy, IgG, IgM, Prevalence

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Introduction

Toxoplasmosis is an infection caused by the protozoan, *Toxoplasma gondii*, which is an obligate intracellular parasite present in many species throughout the world [1]. It attacks a wide range of land and sea animals [2] and various species of birds with its definitive host being the cat.³ It also has a wide variety of intermediate hosts including pigs, dogs, sheep, goat and cattle. Studies have shown that about 5-35% of pork, 9-60% of lamb and 0-9% of beef contain *Toxoplasma gondii* [3] and the consumption of these foods are risk factors of toxoplasmosis. *Toxoplasma gondii* infects a large proportion of the world's population. It is usually asymptomatic in adults, although it may manifest in the form of chorioretinitis, lymphadenitis, or rarely myocarditis and polymyositis.

Toxoplasmosis is a severe disease when acquired in pregnancy and in immuno-compromised persons especially those with defects in T-cell-mediated immunity such as those with haematologic malignancies, bone marrow and solid organ transplant or AIDS [3]. It also attacks the eyes resulting in ocular toxoplasmosis. According to studies on toxoplasmosis; 30% of 30 years old and 10-67% of 70 years old have had a *Toxoplasma gondii* infection [3]. In most developed countries, it is estimated that the seroprevalence of toxoplasmosis in pregnant women of childbearing age is between 4-100% [1] and the incidence of primary maternal infection during pregnancy varies from 1-310 per 10000 pregnancies in Europe, Asia and America [4]. According to Wu [2], the prevalence of congenital toxoplasmosis is 1 in 1000 live births in France. In addition, about 90% of French population, 12.5% of the Japanese population and 60% of the Dutch population are sero-positive for *Toxoplasma gondii* antibodies by the fourth decade of life [2]. In other developed countries like the USA, only 15% of pregnant women reporting at antenatal care are immuned against *Toxoplasma gondii* leaving 85% at risk; implying that toxoplasmosis is high on the public health scale [5].

Research carried out on pregnant women indicated a prevalence of 25% in Bukina Faso [6], 75.2% in the Democratic Republic of Sao Tome [7] and 77.1% in Cameroon [8].

When a woman acquires primary toxoplasmosis in pregnancy, trans-placental transmission to the fetus [2] or transmission to the fetus during vaginal delivery may occur. The clinical presentation of congenital toxoplasmosis in the fetus vary depending on the gestational age. When the infection is acquired by a woman in the first trimester and not treated, approximately 17% of fetuses are infected and may present with conditions such as mental retardation, seizures or severe neurologic defects [3]. It can also result in spontaneous abortion [2]. If acquired in the second trimester and not treated, about 25% of fetuses are infected and it may result in abnormalities such as hydrocephalus or epilepsy. In the third trimester, about 65% of fetuses are infected and may present with symptoms of chorioretinitis [9] or still births [10].

This study was carried out to determine the seroprevalence of *Toxoplasma gondii* specify IgG and IgM antibodies in pregnant women at different gestational ages and to identify the predisposing risk factors for toxoplasmosis in Cameroon.

Methods

Study Area

The research was carried out in Yaounde, the capital city of Cameroon. Yaounde is located between latitude 3.4, 40° north of the equator and longitude 11.1, 120° east of the Greenwich Meridian. It has an altitude of about 760m above sea level. It enjoys two distinct seasons, the dry and the rainy seasons. The annual rainfall and the temperature stand at 15.9mm and 23.5°C respectively. It is metropolitan and cosmopolitan with most of the inhabitants being civil servants and diplomats. There are a lot of recreational facilities such as hotels and sport playing grounds. The population is heterogeneous with about 2,695,600 people and a growth rate of 6.8% per year [11]. Its original inhabitants are the

Ewondos. The development of the town is uneven with a wide gap between the rich and the poor. Most of the poor do not have access to good potable water and have resorted to wells as their only source of drinking water which is not often treated. The social problems of over crowding, poor housing and poor hygienic conditions help in the spread of most parasitic infections. Yaounde has many hospitals including 4 reference hospitals; the General Hospital, the Central Hospital, the Obstetric and Gynecological specialist hospital, and the University Teaching Hospital. There also exist a wide variety of privately owned clinics all of which are striving for a healthy Yaounde. This work was carried out at the Yaounde University Teaching Hospital.

Study population

Pregnant women of ages 18-41 years attending the Yaounde University Teaching Hospital from May to June, 2008 participated in the study. Ethical clearance was obtained from the Regional Delegation of Public Health of the South West Region in Buea, Cameroon. Informed consent was sought and gained from all pregnant women visiting the ante natal clinic for routine pregnancy follow-up visits. Volunteers who fulfilled the eligibility criteria were selected for the study. Each pregnant woman was interviewed using a standardized questionnaire. The questionnaire contained socio-demographic data on age of the women, age of the pregnancy, educational level, occupation, place of abode, source of drinking water, gravidity and parity, type of meat consumed, cat ownership and knowledge of toxoplasmosis.

Specimen collection and laboratory analysis

Venous blood was collected by venepuncture respecting all aseptic techniques. A blood sample of about 3-4ml was collected and rapidly transferred into a labeled dry tube. The samples were transferred to the laboratory and centrifuged at 3000 rpm for 5 min and serum separated from red cells, labeled and stored between 2-8°C for samples that were to be processed 2-7 days from the collection date. Serum samples were analysed using two tests: assay procedures for the determination of *toxoplasma gondii* specific IgG

using immunoComb® ToxolgG and assay procedures for the determination of *Toxoplasma* IgM with “capture” enzyme immunoassay (ELISA) for the determination of IgM antibodies to *Toxoplasma gondii* in human plasma and sera which have been described in detail by Montaya et al [12].

Statistical analysis

The data collection forms were first checked for completeness, obvious errors and inconsistencies which were corrected before they were entered into a computer and double checked. Using Epi-Info, the data were analysed with chi-square and presented as appropriate. The analysis was focused on determining the prevalence of *Toxoplasma gondii* IgG and IgM antibodies in the different trimesters of gestation and the seroprevalence in association with socio-demographic and predisposing risk factors such as age, level of education, occupation, source of potable water, type of regular meat eaten, being in contact with cats and having a history of abortion.

Results

The age range of the women was 18-41 years with a mean of 27.9±5.8 years. Most patients (59.1%) were in their second trimester of pregnancy and the mean gestational age was 4.1 ±0.2 months. Most of the women were housewives or unemployed; 61 (55.5%) of them had at least secondary education (Table 1)

Seroprevalence of toxoplasmosis

Table 2 shows that among 110 blood samples analyzed, 69 (62.7%) {95% CI:53.7-71.7%} were positive for *Toxoplasma gondii* IgG antibodies indicating either acquired immunity or past infection. Three (2.7%) patients were positive for both *Toxoplasma gondii* IgG and IgM antibodies indicating recent infection and 38 (34.6%) were negative for both IgG and IgM. No case of IgM seroprevalence alone was diagnosed implying the absence of an indeterminate case of toxoplasmosis. Table 3 shows the variation of toxoplasmosis with the predisposing risk factors

Table 1: Demographic characteristics of the study participants (n=110)

Demographic characteristic	No (%)
Age group (years)	
10-19	8 (7.3)
20-29	59 (53.6)
30-39	40 (36.4)
≥40	3 (2.7)
Level of education	
None	1 (0.9)
Primary	24 (21.8)
Secondary	61 (55.5)
University/ Higher	24 (21.8)
Occupation	
Unskilled	25 (22.7)
Skilled	26 (23.6)
Unemployed/ housewives	36 (32.7)
Students	23 (20.9)
Term of pregnancy	
First trimester	40 (36.4)
Second trimester	66 (60.0)
Third trimester	4 (3.6)

Table 2: Overall prevalence of toxoplasmosis in pregnancy (n=110)

<i>Toxoplasma gondii</i> serology	No (%)
IgG Positive only	69 (62.7)
IgG and IgM Positive	3 (2.7)
IgG and IgM Negative	38 (34.6)
Total	110 (100)

of age and gestational age. The highest prevalence of 30 (75.0%, 95% CI: 74.7-75.3%) was recorded in the first trimester of pregnancy. The prevalence was high among cat owners. There was no statistically significance difference in terms of knowledge on toxoplasmosis and seroprevalence as well as predisposing risk factors. This seroprevalence was high among those who consumed more than one meat type (76.9%, {95% CI: 69.0-84.8%}) compared to those who consumed one meat type regularly (61.9%, 95% CI: 52.8-71.0%) although the difference was not statistically significant. Table 3 also shows that the prevalence of toxoplasmosis was higher among those who drink untreated water sources and among women with a history of abortion although there was no statistically significant difference with these predisposing risk factors.

Discussion

In this study, the prevalence of *T. gondii* antibodies at different stages of pregnancy was determined and possible risk factors such as owning cats or being in contact with cats, source of potable water, type of meat regularly consumed, knowledge on toxoplasmosis, level of education and history of any abortion investigated. The seroprevalence of *T. gondii* infection in different countries is in the range 15% -77% [13, 14]. In this study, 72 (65.5%) women were indentified as carriers of IgG antibodies to *T. gondii* contrary to 77.1% in the study by Ndumbe et al [8]. This may be due to increase in the general hygienic conditions in the recent past in Yaounde by the Municipal Council. Assob and colleagues (personal communication) discovered a toxoplasmosis seroprevalence of 69.9% in HIV/AIDS pregnant women from the Yaounde Teaching Hospital. We had envisaged that HIV/AIDS patients and their immunocompromised state might be a predisposing factor since toxoplasmosis is an opportunistic infection in HIV/AIDS but this was not the case because these patients were on antiretroviral therapy. In our study, among toxoplasmosis positives cases, 2.7% had both IgG and IgM. However, the presence of IgG antibodies imply exposure because asymptomatic humans can develop very high (>100) titres which may remain elevated for several years or even whole life if repeated exposures are encountered [15].

Although an 8-fold rise in antibody titre, taken two weeks apart, is indicative of a recent infection, this is seldom achieved in practice because by the time patients are seen in the clinic, antibody titres have usually peaked. Compared to IgG antibodies, IgM antibodies are short-lived, and they appear before IgG antibodies [16, 17]. A positive IgG and negative IgM test indicate past contact with toxoplasma antigen, but a positive IgG and IgM test is indicative of recent or current infection. Toxoplasma-specific IgM antibodies

Table 3: Variation of *Toxoplasma gondii* seropositivity with predisposing risk factors in pregnancy (n=110)

Toxoplasmosis risk factors	Total tested No (%)	Positive No (%)	Negative No (%)	χ^2	P-value
Age group(years)				1.03	>0.05
10-19	8(7.2)	5(62.5)	3(37.5)		
20-29	59(53.6)	40(67.8)	19(32.2)		
30-39	40(36.4)	25(62.5)	15(37.5)		
>40	3(2.7)	2(66.7)	1(33.3)		
Trimester of pregnancy				2.7	>0.05
First Trimester	40(36.4)	30(75.0)	10(25.0)		
Second Trimester	66(60.0)	40(60.6)	16(24.2)		
Third trimester	4(3.6)	2(50.0)	2(50.0)		
Proximity to cats				0.92	>0.05
Contact with cats	73(66.4)	50(68.5)	23(31.5)		
No contact with cats	37(33.6)	22(59.5)	15(40.5)		
Knowledge on toxoplasmosis				4.36	>0.05
Had knowledge on toxoplasmosis	16(14.5)	14(87.5)	2(12.5)		
Had no knowledge on toxoplasmosis	94(85.5)	58(61.7)	36(38.3)		
Meat consumption				2.14	>0.05
One meat type	84(76.4)	52(61.9)	32(38.1)		
More than one meat type	26(23.6)	20(76.9)	6(23.1)		
Sources of potable water				1.63	>0.05
Tap(treated)	95(86.4)	61(64.2)	34(35.8)		
Well(untreated)	12(10.9)	8(66.7)	4(33.3)		
Stream(untreated)	1(0.9)	1(100)	0(0.0)		
Others	2(1.8)	2(100)	0(0.0)		
History of abortion				1.37	>0.05
Abortion	31(28.2)	23(74.2)	8(25.8)		
No abortion	79(71.8)	49(62.0)	30(38.0)		

may be detected by ELISA for 6-12 months but rarely as long as 18 months after acute acquired infection [18].

Most of the study population (53.6%) belonged to the age group 20-29 years corresponding to peak fertility at this period of reproductive life. The prevalence of *T. gondii* increased with age [13] but in this study, no effect on age and seropositivity was observed. The reason for increased prevalence with age might be increasing risk of exposure with age.

There was a controversial increase in the frequency of antibodies with respect to knowledge on toxoplasmosis. Majority of women who had some knowledge on toxoplasmosis were either in the health sector or had university education implying that they might have known of toxoplasmosis ever before they became

informed of it. It should be noted that the prevalence of serum antibodies against toxoplasmosis varies throughout the world and depends on eating habits, hygiene and climate [2].

Seroprevalence was found to differ according to usage of different potable water sources in the study although it was not statistically significant. Drinking untreated water sources can be a risk factor, as these water sources are often contaminated with wastes including cats faeces. The high seroprevalence in pipe-borne water users may be in accordance with articles that have shown the presence of oocysts in chlorinated water [1]. Bowie et al [19] investigated an outbreak in the western Canadian province of British Columbia and found that the municipal water system that used unfiltered, chloraminated

surface water was the likely cause of the large community-wide epidemic of toxoplasmosis.

Frequent consumption and type of meat (pig, sheep and goat) have been indentified as the principal predisposing risk factors for *Toxoplasma gondii* infection in several studies in humans [13,20,21]. No relationship was established between seroprevalence and type of meat consumed in this study probably due to the fact that people usually consume well-cooked meat. In Yaounde, beef and pork are commonly consumed especially roasted meat locally called 'soya'. The cooking temperature of meat is an important factor in the infection of *Toxoplasma gondii*. Thorough cooking can limit the spread of *Toxoplasma gondii*.

The association of cats and human toxoplasmosis is difficult to assess by epidemiological surveys because soil, not the cats, is the main culprit. Oocysts are found on cat fur [20] and more often buried in soil along with cat faeces, and soil contact is universal and difficult to avoid [22]. In Yaounde, the cat is a common stray domestic animal and there is always a possibility that oocysts of the stray infected cats may gain infectious ability outside the animal. In this study, no relation was established between toxoplasmosis, and cat ownership or being in contact with cats. In previous studies, lower educational level, soil-related occupations [14], eating raw or unwashed vegetables or fruits [23], having poor hand hygiene [14], and contact with soil, were all found to be predisposing risk factors for toxoplasmosis.

There were some limitations to this study. The assessment of risk factors was done according to information given by the women many of whom might have suffered from recall bias. Some issues such as types of meat consumed, potable water and being in contact with cats might be misrepresented as higher because the women might have been shy to associate themselves to bad habits.

Conclusion

Soil-related occupations, eating raw or unwashed vegetables or fruits, having poor hand hygiene, eating raw or undercooked meat and cat ownership are potential predisposing risk factors to toxoplasmosis. All pregnant women should be screened for toxoplasmosis. Toxoplasma IgG and IgM seronegative pregnant women should be monitored throughout pregnancy to ensure that they don't acquire and develop acute toxoplasmosis. Health personnel in charge of pregnant women should sensitize them on predisposing risk factors to toxoplasmosis.

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Conflict of interest

There are no conflicts of interest associated with this work.

Contributions of authors

We declare that this work was carried out by us and all liabilities pertaining to claims relating to the content of this article will be borne by us. DSN participated in the design, analysed the data, drafted the manuscript and substantially revised it, ALN designed the study, contributed in the write-up and substantially revised the manuscript, JCNA and HLFK participated in the design, write-up and substantially revised the manuscript for academic content, RTT participated in the design, data collection and write-up, and EAA designed the study, supervised it, participated in the write-up and substantial revision. All authors read and approved the manuscript.

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