



Sero-epidemiology of Toxoplasmosis among Pregnant Women in the University of Port Harcourt Teaching Hospital, Nigeria

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ABSTRACT

Background: The objective of this study was to determine the sero-prevalence of *Toxoplasma gondii* infection and associated risk factors among pregnant women in the University of Port Harcourt Teaching Hospital, Nigeria.

Methods: A cross-sectional study involving two hundred and eighty-eight (288) pregnant women who gave informed consent was done. Questionnaires were administered to determine their socio-demographic and risk factors. Enzyme-linked immunosorbent assay (ELISA) was performed on all patients' sera to detect anti-*Toxoplasma* Immunoglobulin G (IgG) and M (IgM). Data was analyzed using the statistical package Epi info version 6.04d.

Results: A total of one hundred and eighty-nine women (65.6 %) were sero-positive for IgM and/or IgG. Thirty-three (11.5%) were positive for IgM only, one hundred and twenty-three (42.4%) for IgG only and thirty-three (11.5%) for both. Consumption of beef was a statistically significant risk factor associated.

Conclusion: A significant proportion of pregnant women have been exposed to *Toxoplasma gondii*, with the risk factor significantly associated with infection being consumption of beef, which is a very common practice in Port Harcourt. It is therefore necessary to heighten enlightenment of the populace particularly women of child-bearing age and pregnant women on toxoplasmosis-specific preventive practices to reduce their exposure to the pathogen and its potential complications. Routine screening for Toxoplasmosis in pregnancy would be largely beneficial in its prevention and control.

Keywords: *Toxoplasma gondii*; Pregnancy; ELISA, Risk factors

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii*, an obligate intracellular parasite of many species of animals throughout the world, which causes a variety of clinical syndromes in humans and animals.^{1,2}

Toxoplasmosis appears to be more prevalent in hot, humid climates^{2,3} such as we have in Nigeria. Felidae (members of the cat family) are the definitive hosts of the parasite while many other mammals and birds serve as intermediate hosts.²

Toxoplasmosis is important medically, socially and financially, due to its serious implications in pregnant women, congenitally infected fetuses and immuno-suppressed individuals, as such it represents a significant public health problem.⁴



About 20% to 90% of the world's adult population in different regions is reported to have had contact with the parasite but only about 10% of infected individuals develop clinical signs and symptoms.^{5,6,7}

In various parts of Nigeria, seroprevalence rates of toxoplasmosis among pregnant women ranging from 29.1%^{8,9} to 75.4% have been reported^{10,11}. Moreso, in Port Harcourt, a high seroprevalence of toxoplasmosis among domestic cats in Port Harcourt and significant positivity for fecal oocysts was recorded.¹²

The rate of transmission of *T. gondii* from an infected pregnant woman to her fetus is 10-15% in the first trimester of gestation and may increase to 68% in the third trimester¹³. Thus maternal infections early in pregnancy are less likely to be transmitted to the foetus than infections acquired later in pregnancy. However, early foetal infections are likely to have more severe consequences than late infections. Therefore, the severity of foetal disease varies inversely with the gestational age at which maternal infection occurred.¹³

Infection during pregnancy can cause spontaneous abortion, and a range of other disorders including intra-uterine foetal death, hydrops foetalis, amniotic fluid disorders, hydrocephalus and neurological disorders such as chorioretinitis, blindness and mental retardation in congenitally infected newborns. Although most infected foetuses (approximately 75%) do not have obvious clinical signs at birth, many (approximately 80-85%) are likely to have manifestations later in life.^{13,14}

It is even more worrisome to note that immunosuppressed pregnant women such as human immunodeficiency virus (HIV) infected pregnant women, can transmit infection to their babies even when they got infected prior to pregnancy^{15,16}. This finding is significant and has implications in a place like Port Harcourt where a high HIV prevalence rate of 7.3% was reported among pregnant women.¹⁷

Toxoplasmosis is the most common cause of intraocular inflammation in the world.⁴ Ocular toxoplasmosis was found to account for 19% of all chorioretinal scars suspected to be of infectious origin and 56% of all school admissions into the school for the blind, being only second to congenital cataracts⁴. It is also a frequent cause of encephalitis in severely immunosuppressed patients with Acquired Immunodeficiency Syndrome (AIDS).¹

Serology for the detection of *Toxoplasma*-specific antibodies is the primary diagnostic method to determine infection with *Toxoplasma*. Enzyme-linked immunosorbent assay (ELISA) one of the most commonly used of these, is widely available, easy to perform and not time consuming. A positive IgG titer indicates infection with the organism at some time. On the other hand, a negative IgM test essentially excludes recent infection.

Documented risk factors for toxoplasmosis include contact with infected cat litter, outdoor farming, drinking of untreated water, consumption of improperly cooked meat of infected animals including beef, mutton, pork and goat milk.^{6,8,9}



Maternal toxoplasmosis as a risk factor for negative pregnancy outcomes was investigated and infection was found to be much more frequent in women with habitual abortion and still births than in the normal pregnancy group.^{6,13}

Routine screening for toxoplasmosis among pregnant women receiving antenatal care is not done in Nigeria but this is the practice in some European countries including France and Austria, which are countries with a high burden of toxoplasmosis.¹⁸ The need for routine screening for toxoplasmosis in pregnancy is probably due to the grave medical, psycho-social, financial and economic consequences of infection with *Toxoplasma gondii*. It is thus important that studies which evaluate the seroprevalence of toxoplasmosis should be done in Nigeria in order to establish a baseline seroprevalence which will produce the data to justify the need for routine screening in pregnancy.

This study was carried out to determine the seroprevalence of *Toxoplasma gondii* infections, its associated risk factors and association with past history of negative pregnancy outcome among pregnant women in Port Harcourt, Nigeria.

METHODOLOGY

A cross-sectional study carried out in 2013 in the departments of Medical Microbiology and of Obstetrics and Gynaecology of the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers state, Nigeria.

The Study Population was made up of pregnant women registered at the antenatal clinic. They were consecutively recruited at the point of first visit until sample size was achieved.

Ethical approval was obtained from the Ethical committee of the hospital and informed written consent from the study participants.

Well-structured questionnaires written in simple and clear English were self-administered, except for women who couldn't read and write in which case it was interviewer-administered. Five milliliters (5 ml) of venous blood was obtained using a sterile serum vacutainer needle and bottle, taken to the laboratory within one hour of collection, where tubes were centrifuged at 2500revs per minute for 3 minutes. Clear sera was carefully collected into sterile tubes and stored frozen at -20°C until tested.

Each sample was tested for the presence of anti-*Toxoplasma* antibodies, IgG and IgM, using commercial ELISA Kits (Diagnostics Automation Inc. / Cortez diagnostics, USA) following manufacturer's instructions.

Access to all personal data and samples of the research participants was limited to the Investigator, Supervisors and Research assistants where necessary.

Data obtained from this study was analyzed using the statistical software package, *Epi-Info version 6.04d*, (CDC, USA 2001). The level of significance was set at 0.05. Odds ratio was used to estimate the possible association between seropositivity and the past history of negative pregnancy outcome as well as the following risk factors: possession of cats, presence of cats in neighbourhood, outdoor gardening/ farming, use of gloves/ not for farming, type of meat most commonly consumed, the preferred cooking method and source of drinking water.



RESULTS

Table 1 shows the socio-demographic distribution of the study participants. The minimum age was 22 years and maximum 46 years, with the highest number of participants, 198(69.5%) lying between 25-34 years.

The minimum gestational age was four (4) weeks and the maximum was thirteen (13) weeks however, the highest number of patients was at eleven (11) weeks gestational age (Table 1).

One hundred and sixty-five (57.3%) participants, being the majority, lived in urban areas while one hundred and two (35.4%) and twenty-one (7.3%) lived in semi-urban and rural areas respectively (Table 1). Most of the women (68.8%) had tertiary education (Table 1).

Figure 1 shows that only a minority of women (3.4%) had heard the term Toxoplasmosis before the time of this study.

One hundred and five women (36.5%) had previous miscarriages and/or stillbirths ranging from 1 to 3. Sixty (57.1%) had 1, thirty (28.6%) had 2 and fifteen (14.3%) had 3 previous miscarriages and/or stillbirths. Of these, Seventy-two (25.0%) were IgG seropositive while thirty-three (11.5%) were seronegative (Table 2). On the other hand, out of one hundred and eighty-three women (63.5%) who had no history of negative pregnancy outcomes, one hundred and sixteen (40.3%) were seropositive while sixty-seven (23.3%) were sero-negative (Table 2).

Following the IgG and IgM ELISA assays, a total of one hundred and eighty-nine (65.6 %) samples were sero-positive for IgG and/or IgM, in various combinations. Thirty-three samples (11.5%) were positive for IgM only, one hundred and twenty-three (42.4%) were positive for IgG only while thirty-three (11.5%) were positive for both IgM and IgG. Six samples had equivocal results for IgM. Ninety-nine (34.4%) were negative for both antibodies.

Possible association between the risk factors assessed using questionnaires and *Toxoplasma gondii* infection was measured using Odds ratio. Consumption of beef was significantly associated with seropositivity for *Toxoplasma gondii* whereas though there was a positive association between seropositivity for toxoplasmosis and other risk factors evaluated which include Possession of cats, having cats in one's neighborhood, outdoor farming, and consumption of half-cooked meat / tasting of meat before it is cooked, no statistically significant association was found (Table 3). Drinking of sachet water (OR 0.62, 95% CI 0.36-1.07, p=0.09) and borehole water (OR 0.30, 95% CL 0.17-0.53, p=0.001) showed no likelihood of increasing the risk of infection.

Only nine women (3.4%) had previous knowledge of toxoplasmosis while the other participants (96.6%) had no previous knowledge or didn't respond (Figure 1).



TABLE 1. Socio-demographic characteristics of pregnant women

DEMOGRAPHIC FACTOR	FREQUENCY N (n%)
Age Group	
15-24	9 (3.1)
25-34	198 (68.8)
35-44	72 (25.0)
45-54	6 (2.1)
No response (Blank)	3(1.0)
Total	288 (100.0)
Residential Area	
Urban	165 (57.3)
Semi-Urban	102 (35.4)
Rural	21 (7.3)
Total	288 (100.0)
Educational Status	
Primary	12 (4.2)
Secondary	69 (23.9)
Tertiary	192 (66.7)
No response (Blank)	15(5.2)
Total	288 (100.0)

KEY: N= Number of participants; n= Percentage value

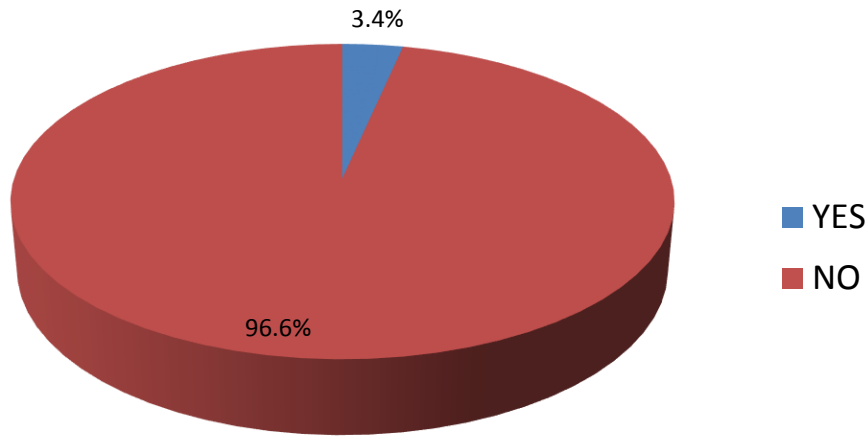


Fig 1. Knowledge of Toxoplasmosis

TABLE 2. Relationship between IgG anti-*Toxoplasma gondii* sero-positivity and history of negative pregnancy outcome

PREVIOUS MISCARRIAGE/ STILLBIRTH	IgG POSITIVE N (n%)	IgG NEGATIVE N (n%)	TOTAL N(n%)	ODD'S RATIO (95% CI)	P VALUE
YES	72(25.0)	33 (11.5)	105(36.5)	1.26 (0.73-2.18)	0.447
NO	116 (40.3)	67 (23.3)	183 (63.5)		

KEY: N= Number of participants
n= Percentage value



Table 3. Relationship between Seropositivity and risk factors among Pregnant women

Risk Factor	No of women	Seropositive (n%)	Odds ratio (95% CI)	P-value
Consumption of beef				
Yes	206	120 (41.7)	1.78	0.038
No	82	36 (12.5)	(1.03-3.09)	
Tasting of meat while uncooked				
Yes	96	51 (18.1)	1.00	0.913
No	186	99 (35.1)	(0.59-1.68)	
Possession of cats				
Yes	15	12 (4.2)	3.58	0.07
No	273	144 (50.0)	(0.91-11.74)	
Presence of cats in Neighbourhood				
Yes	75	45 (16.5)	1.33	0.369
No	198	105 (38.5)	(0.75-2.36)	
Possession of outdoor farm/garden				
Yes	45	24 (8.3)	0.96	0.968
No	243	132 (45.8)	(0.56-1.91)	

KEY: CI = Confidence interval, n = percentage value



DISCUSSION

The level of knowledge of toxoplasmosis among pregnant women in Port Harcourt is very low as only 3.4% of our study participants indicated having heard of toxoplasmosis even when the majority of the participants had tertiary education and lived in urban areas (69.2% and 57.7% respectively). This indeed is the case in some other settings where a poor knowledge about the disease was observed not just among pregnant women but also among their antenatal care givers.^{20,19,20} Education of women has been shown to reduce the incidence of the disease and so it is necessary for obstetricians and primary care givers to adequately educate pregnant women and indeed all women of child-bearing age and female adolescents on the risk factors, sources of infection and preventive measures.^{21,22}

A history of previous miscarriage(s) and stillbirth(s) which are two negative pregnancy outcomes that have been associated with *Toxoplasma* infection in pregnancy were higher among seropositive women, however no significant statistical association was found between IgG seropositivity and the history of previous miscarriage(s) and stillbirth(s). This is similar to the finding by Adesiyun and co-workers.¹³

This may be due to the fact that these women had got infected well before the pregnancy and thus the miscarriages and stillbirths they had were probably due to conditions other than toxoplasmosis. World- wide, prevalence rates of toxoplasmosis in women with abnormal pregnancies and abortions is said to vary from 17.5% to 52.3%²³.

Consumption of beef was significantly associated with seropositivity in this study. In Port Harcourt, pork or lamb aren't commonly consumed unlike in Northern Nigeria and Europe, so most of our study participants ate beef mostly while none reported eating pork or lamb most, probably due to geographical and cultural differences. A study in France showed the consumption of beef to be the sole predictor of sero-conversion during pregnancy while another study the handling and consumption of raw or improperly cooked beef, lamb and other types of meat as predictors of sero-conversion in pregnancy.²⁴

The tasting of meat while cooking and the consumption of lightly cooked meat showed no significant association with seropositivity in this study. This is probably because most women now cook their meat properly following increased knowledge of the dangers of consuming improperly cooked meat even though not necessarily specific for toxoplasmosis prevention and also the common practice of boiling meat until it is soft before consumption. This finding differs from that in Zaria⁹ which showed infection to be 1.9 times more likely to occur in women with the habit of tasting meat while cooking and seropositivity for toxoplasmosis to be significantly associated with tasting of meat while cooking, however, in a European study the practice of tasting meat while cooking was of borderline importance.^{5,25} This therefore suggests that these women probably got infected in the process of handling (possibly in the abattoirs and market places where beef is purchased) and preparing of beef (such as infrequent washing of knives and chopping boards after cutting raw meat) before cooking.



In this study, possession of cats and having cats in one's neighbourhood were not significant risk factors for seropositivity. This is similar to other studies in Lagos Nigeria, Ghana and other parts of the world.^{6,8,25} Furthermore, Ishaku and co-researchers working in Zaria, Nigeria reported no significant association between contact with cats and infection.⁹ This is consistent with the observation that food-borne transmission of *T. gondii* is increasingly recognized as a potentially more important source of infection to humans than cats in many endemic areas.^{5,24}

Outdoor gardening, especially with bare hands was a predictor of seropositivity in this study and a number of others, such as a large multicentre study in Europe where it was found that 17% of their infections were due to soil contact.²⁴ **Error! Bookmark not defined.** All our study participants who admitted working on outdoor gardens or farms did so with bare hands and 55.6% of them were seropositive.

Drinking of sachet and borehole water showed no likelihood of increasing the risk of infection. This probably indicates increased awareness of the need for treatment/ purification of drinking water and increased effectiveness of the regulatory bodies concerned with production of drinking water for commercial purposes in this environment.

CONCLUSION

A significant proportion of pregnant women have been exposed to *Toxoplasma gondii*, with the risk factor significantly associated with infection being consumption of beef which is a very common practice in Port Harcourt. It is therefore necessary to heighten enlightenment of the populace particularly women of child-bearing age and pregnant women on toxoplasmosis-specific preventive practices to reduce their exposure to the pathogen and its potential complications. Routine screening for Toxoplasmosis in pregnancy would be largely beneficial in its prevention and control.

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