



Comparative Seroprevalence and Risk Factors of Toxoplasmosis among Four Subgroups in Port Harcourt, Nigeria

Onosakponome Evelyn Orevaoghene^{1*} and Michael Ndubuisi Wogu²

¹*Pamo University of Medical Sciences, Port Harcourt Nigeria.*

²*University of Port Harcourt, Choba, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Author OEO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MNW managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was carried out to investigate the comparative seroprevalence and associated risk factors of toxoplasmosis among four subgroups in Port Harcourt viz: immunocompromised persons (HIV), pregnant women (PTW) and schizophrenics (SZN), using immunocompetent persons (IP) as controls.

Study Design: A descriptive cross-sectional study was adopted.

Place and Duration of Study: The study was carried out in the University of Port Harcourt Teaching Hospital (UPTH), Braithwaite Memorial Specialist Hospital (BMSH) and Neuro Psychiatric Hospital (NPH) between March 2016 and February 2017.

Methodology: A total of 800 (200 from each of the group) samples were collected from subjects from the three hospitals. Study participants of ≥ 20 years were used in this study. The detection of *T. gondii* antibodies in examined sera was carried out using ELISA –IgG and IgM assay using standard procedures. Structured questionnaires were used to collect data on social demographic risk factors associated with toxoplasmosis.

*Corresponding author: E-mail: eonosakponome@pums.edu.ng;

Results: Out of the 800 (200 per group) examined, SZN recorded the highest seroprevalence of IgG 50%(100)($P=.05$) and IgM 4%(8) followed closely by HIV subjects with seroprevalence of IgG 32%(72) ($P=.05$) and IgM 2%(3). However, the seroprevalence were not significantly different from that of the immunocompetent control group which had the least seroprevalence. Schizophrenia subjects in age group 20-24 years recorded the highest seroprevalence with ($P=.05$) The unemployed recorded the highest seroprevalence for SZN with rates of 43% for IgG. More males were infected with the disease than females. Male Schizophrenics recorded a seroprevalence of 20.9%. There was no significant relationship between seroprevalence and demographic factors among subjects.

Conclusion: Our findings indicated that toxoplasmosis was relatively prevalent ($P=.05$) among the studied population, with schizophrenics having the highest seroprevalence. Therefore, incorporating detection of anti-*Toxoplasma* antibodies in routine testing in our health care centers is recommended for monitoring and control of the disease.

Keywords: *Toxoplasma gondii*; seroprevalence antibodies; schizophrenics; toxoplasmosis.

1. INTRODUCTION

Toxoplasmosis is an important but neglected tropical parasitic infection with global distribution and significance [1,2]. It is caused by the protozoa called *Toxoplasma gondii* [2]. About a third of the world's human population is estimated to harbor a *Toxoplasma* Spp., infection [3]. The Centre for Disease Control and Prevention (CDC) classifies toxoplasmosis as one of the neglected tropical diseases [2,3].

Several studies have shown that cats and other feline species are the definitive host as they are the only animals that excrete the oocysts into the environment [1,3]. This concedes to the fact that cohabiting with cats increase the chances of getting infected. However, direct infection through handling cats is believed to be very rare [2,3,4]. Some animals including humans may also serve as intermediate hosts in which the parasite may cause systemic infection that results in the formation of tissue cysts [2]. Transmission can also occur through ingestion of raw or partly cooked meat especially pork, lamb or venison containing toxoplasma cysts. Oocysts may also be ingested through knives, utensils or cutting boards contaminated by raw meat [2,4] and through ingestion of oocysts shed by cats in the environment. *Toxoplasma* Spp., could also be transmitted by transplacental means or through organ transplantation [3,4].

In humans with a normal immune competence, *Toxoplasma* Spp., infections tend to be asymptomatic in most cases. This is not the case in individuals who are immunocompromised or pregnant as toxoplasmosis can lead to serious pathological and congenital adverse effects [4,5,6]. Toxoplasmosis could be severe and life-

threatening during pregnancy, especially to fetus and new born babies [4,6]. One of the late indicators of congenital toxoplasmosis is Chorioretinitis [6]. Vertical transmission occurs, causing mental retardation, blindness, epilepsy and death [7]. Recent research has also linked Toxoplasmosis with attention deficit hyperactivity disorder, obsessive compulsive disorder and schizophrenia [7,8,9]. Numerous studies found a positive correlation between latent toxoplasmosis and suicidal behavior in [7,8].

Toxoplasmosis can be severe and life threatening to immunocompromised patients causing severe encephalitis through acute infections or reactivation of latent infection [3,10,11].

The geographical distribution of this disease depends on regions and weather conditions where the parasite survives in the environment [3,10]. It is estimated that between 30% and 65% of all countries harbor the parasite [3,4]. Human toxoplasmosis is reported to be widespread in Sub-Saharan Africa with a seroprevalence of 3.6–84% in different countries [12,13,14,15,16]. The variation in the prevalence rates is attributed to the environmental and socio-cultural factors. High prevalence rates of 74.7% was reported in Ethiopia [15], 66.6% in central African republic and 59.4% in republic of congo [16] while in sudan and South Africa about 29.4% and 21.5% respectively are carriers [4,17]. In Nigeria seroprevalence of 32%, 23.9% and 22.2% have reported in Zaria, Maiduguri, and Abuja respectively [12,13,18]. However in the Niger-delta region especially Port Harcourt, there is no research on toxoplasmosis in schizophrenics which is currently an emerging global health concern. Currently, *Toxoplasma*

Spp., testing is not carried out in government owned health institutions in Nigeria. The aim of this study is to determine the seroprevalence of toxoplasmosis among four physiologically distinct groups viz: Immunocompetent subjects, immune-compromised subjects (HIV patients), pregnant women and individuals with schizophrenia.

2. MATERIALS AND METHODS

2.1 Study Population

This prospective comparative study was carried out on a study population comprised of 800 people attending the tertiary medical facilities; University of Port Harcourt Teaching Hospital (UPTH), Braithwaite Memorial Specialist Hospital (BMSH) and Neuro Psychiatric Hospital (NPH) from March 2016 to February 2017. Four sub-groups of physiologically distinct individuals were sampled for the study. These include immunocompetent subjects (controls), Immunocompromised subjects, Pregnant women and Schizophrenia individuals. A total of 200 samples were collected from each of the groups from both hospitals. The technique adopted for sampling was random sampling. Study participants of ≥ 20 years were used in this study.

2.1.1 Inclusion criteria

All pregnant women must test positive to pregnancy test and negative to HIV I and II tests.

All healthy controls must test negative to HIV and pregnancy tests.

All HIV female patients must test negative to pregnancy test.

All schizophrenics must test negative to HIV and pregnancy test.

2.1.2 Exclusion criteria

All those who refuse to give oral/written consent.

All those who gave oral/written consent but were not eligible based on preliminary test results.

2.2 Detection of Anti-Toxoplasma IgG and IgM Antibodies

Five millimeters of venous blood was collected from each of the 800 participants. Blood samples were allowed to clot and then centrifuged for 5 minutes at (1500) rpm. Serum was collected and stored at 2°C. The developing plates cards,

reagents and specimen were all brought to temperature of 24°C. 800 participants were subjected to Pregnancy and HIV tests to determine exclusion and inclusion criteria using Lab Acon pregnancy kit and Alere kit for HIV Tests, before *Toxoplasma* Immunoglobulins IgG and IgM antibody assays were carried using BioCheck for *Toxoplasma* Immunoglobulins IgG and IgM enzyme immunoassay test kit (ELISA) following standard methods recommended by the producer.

2.3 Statistical Analysis

Data generated from the work was analyzed using descriptive statistics (frequencies, percentages and means), two-way analysis of variance (two-way ANOVA) and mean separation. Software packages (SAS) version 9 and Microsoft Excel were used for the statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Results

In this study, 276(34.4%) - IgG and 30(3.8%) - IgM of the 800 study participants examined showed the presence of *T. gondii* antibodies in the blood with schizophrenics being most infected with a seroprevalence of 100 (50%) and 8(4.0%) for IgG ELISA and IgM ELISA test respectively. This was followed closely by HIV subjects' with seroprevalence of 72(36%) test and 3(2.0%) for IgG ELISA and IgM assay test respectively. The immunocompetent subjects recorded the least 43(22%) for IgG ELISA *T. gondii* antibody tests. The two-way ANOVA showed that the seroprevalence for each of the groups were significant, (P=.05) for IgG ELISA tests while it was insignificant for IgM ELISA tests [Table 1].

Schizophrenics recorded the highest seroprevalence for IgG *Toxoplasma* antibodies for 20-24 yrs 21(18.8%). The ANOVA statistical results showed that the various seroprevalence rates for IgG based on age were insignificant at (P=.01). However, the seroprevalence for IgM ELISA based on age was significant.

Schizophrenics also had the highest seroprevalence of toxoplasmosis among the unemployed with a seroprevalence of IgG 34(43%) and IgM 2(2.5%). HIV patients recorded the highest seroprevalence for traders' 38 (17.2%) for IgG ELISA *Toxoplasma* Spp., tests.

There was no significant difference in the seroprevalence between individuals in the various occupation groups [Table 1].

Schizophrenics showed the highest seroprevalence among males recording 57 (20.9%) for *Toxoplasma* Spp., IgG ELISA assay tests. This was statistically significant compared to the other subgroup's HIV subpopulation recorded a lower seropositivity to *T. gondii* infection 44(8.2%) [Table1].

The highest seroprevalence was recorded for subjects that do not wash fruits and vegetables properly or do not wash fruits and vegetables at all before consumption. Subjects who had a history of owning pets recorded the least seroprevalence all the sub groups. There was no significant difference between the various risk factors as relates to the tests and subgroups of subjects [Table 2].

3.2 Discussion

The study showed that the schizophrenics had the highest seroprevalence IgG 100(50%), IgM 5(2.5%) followed closely by immunocompromised HIV persons IgG 72 (36%), IgM 3(1.5%). Immunocompetent persons (IP) and pregnant women recorded slightly lower prevalence of 61(30.5%) and 43(21.5%) for IgG respectively. Recent studies revealed that levels of antibodies to *T. gondii* have been found to be increased in individuals with schizophrenia as compared to controls with an odd ratio for *Toxoplasma* Spp. seropositivity between 2.4-4.4 [8,9,19,20]. Many reports revealed that *Toxoplasma* Spp. might represent a major pathogen in some cases of psychosis. It has been proven that the parasite infection could increase the dopamine level in mice brains [8,19]. Dopamine plays a key role in psychosis is cases such as schizophrenia [8,20]. The seroprevalence of *T. gondii* in this study in relation to (IgG 50%, is similar to reports from a study carried out by [13,20], in which the seroprevalence of the toxoplasmosis was reported to be 34%. Varying seroprevalence values of 12.4% [7]; 72.5% [14]; 85.7% [9,20], have also been reported for IgG *Toxoplasma* Spp., antibodies in schizophrenics. These varying seroprevalence rates may be due to environmental conditions, level of individual hygiene, social custom and habits.

In 2014, Smith Gary in attempting to prove the link of the sampled individuals between *T. gondii* with schizophrenia was able to determine the proportion of schizophrenia cases attributable to *T. gondii* infection, by calculating the population attributable fraction (PAF); a measure used by epidemiologists to understand the importance of a risk factor. The PAF was 21.4 for an average life time. This means that a fifth of all schizophrenia cases over a lifetime could be prevented by stopping *T. gondii* infections from occurring. Invariably, the higher the prevalence of *T. gondii* in a city, the higher the prevalence of schizophrenia [19].

The seroprevalence of toxoplasmosis in HIV patients in this study 36% IgG is similar to reports of other studies of seroprevalence rates of 38.1% in Mashhad and 32.4% in Zaria Nigeria [10,11,13]. Other similar studies reported varying seroprevalence including 22.2% in Abuja, Nigeria, 96.3% in Mazamdaran, Iran [13,18,21]. The IgM antibody response to *Toxoplasma* Spp infection is short-lived and it is frequently suppressed to undetectable levels in settings of immunosuppression. In agreement, our study revealed lower levels of IgM seropositivity 2% (3) compared to IgG 36% (72) similar observation of low levels of IgM compared to IgG seropositivity in HIV patients have been reported by Imam et al. in similar studies from South Africa [17] Northern Nigeria [11,18], Mexico [7] These low values of IgM antibodies in HIV positive subjects lends support to the view that the screening for this antibody in routine diagnosis of toxoplasmosis in non-pregnant HIV infected persons may be of limited value [11,13,18,21].

The seroprevalence of *T. gondii* for pregnant women in this study was reported to be 61(30%) IgG and (5) 2% IgM. This agrees with the findings of some other similar studies where overall prevalence was, found to be 25.5% in Sudan, 29.4% in Mekkah, 21.3% in Almadina, 34.1% in Burkina Faso [14,17,22,23]. Different seroprevalence rates were reported in pregnant women from other studies as well. A study carried out in Abuja, Nigeria reported a seroprevalence of 44.7% and another study carried out in Ethiopia reported a higher seroprevalence of 64% [23]. The causes of the variation in seroprevalence are attributed to environmental distinctiveness, prevailing risk factors and mother hygiene levels [2,3].

Table 1. Seroprevalence based on socio- demographic factors among the study population

Factors /Parameters	Number examined	Number positive (%)							
		Ip		HIV		PTW		SZN	
		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Age group									
20-24	112	4(3.6)	3(2.7)	8(7.1)	1(0.9)	6(5.4)	2(1.8)	21(18.8)	3(2.7)
25-29	150	15(10.0)	2(1.3)	13(8.7)	1(0.6)	12(8.0)	1(0.6)	14(9.3)	1(0.6)
30-34	209	11(5.3)	4(1.9)	18(8.6)	0(0.0)	23(11.0)	1(0.5)	21(10.0)	1(0.5)
35-39	171	8(4.7)	4(2.3)	13(7.6)	1(0.6)	17(9.9)	2(1.2)	22(12.9)	0(0.0)
>40	158	5(3.2)	1(0.6)	20(12.7)	0(0.0)	4(2.5)	2(1.3)	22(13.9)	0(0.0)
Occupation									
Artisans	110	5(4.5)	3(2.7)	11(10.0)	1(0.9)	9(8.2)	1(0.9)	6(5)	0(0.0)
Civil servants	111	5(4.5)	1(0.9)	5(4.9)	0(0.0)	13(11.7)	2(1.8)	2(1.8)	0(0.0)
Farmers	25	0(0.0)	1(4.0)	1(4.0)	0(0.0)	1(4.0)	0(0.0)	2(8.0)	0(0.0)
Students	185	12(6.5)	3(1.6)	6(3.2)	1(0.5)	9(4.9)	1(0.5)	43(23.2)	2(1.1)
Teachers	69	8(11.6)	1(1.4)	4(5.8)	0(0.0)	5(7.2)	1(1.4)	3(4.3)	0(0.0)
Traders	221	13(5.9)	5(2.3)	38(17.2)	1(0.5)	20(9.1)	2(0.9)	10(4.5)	1(0.5)
Unemployed	79	0(0.0)	0(0.0)	7(8.9)	0(0.0)	5(6.3)	1(1.3)	34(43.0)	2(2.5)
Sex									
Male	273	17(6.2)	6(2.2)	28(10.2)	2(0.7)	0(0.0)	0(0.0)	57(20.9)	5(1.8)
Female	534	26(4.9)	1(1.5)	45(8.4)	1(0.2)	61(11.4)	10(1.9)	43(8.5)	3(0.6)
Overall	800	43(21.5)	14(7.0)	72(36.0)	3(1.5)	61(30.5)	8(4.0)	100(50.0)	5(2.5)

IP= Immunocompetent Persons; HIV= Hiv Positive Persons; PTW = Pregnant Women; SZN = Schizophrenics
 IgG= Immunoglobulin G-toxoplasma ELISA test; IgM= Immunoglobulin M-toxoplasma ELISA test; (P=0.9044) (P<0.05)

Table 2. Seroprevalence of toxoplasmosis among the study population based on risk factors

Risk factors		NE	IgGE (NP%)	IgME (NP%)	Mean separation
Engage in farming	yes	440	128(24.5)	13(2.0)	1.68a
	no	360	148(33.6)	17(4.4)	1.38a
Wash fruits	yes	294	71(20.7)	12(2.4)	0.42b
	no	506	205 (33.5)	18(3.3)	0.89a
Drink treated water	yes	227	66(12.3)	6(1.8)	1.13b
	no	573	210(34.8)	24(2.6)	1.88a
History of owning pets	yes	269	96(28.7)	11(2.2)	0.80a
	no	531	180(29.3)	19(3.6)	0.75a
Consume suya	yes	168	65(11.3)	13(4.2)	0.67a
	no	639	211(33.0)	19(2.7)	1.05a

Means that do not share a letter (a or b) are significantly different $P = .05$ ($P = .05$); IgG= Immunoglobulin G-toxoplasma ELISA test; IgM= Immunoglobulin M-toxoplasma ELISA test; NE = Number Examined; NP = Number Positive

Toxoplasmosis among immunocompetent subjects being the control group was found to be 43 (21.5%) IgG and 14 (7%) IgM. This supports the fact that latent toxoplasmosis is asymptomatic [7,17,18,19]. Similar seroprevalence rate of 22.2% and 20% were reported in similar comparative study in Abuja and Eastern Nigeria respectively [17,18].

Schizophrenics within the age group of 20-24 recorded the highest seroprevalence. This age group mainly consists of Youths of school age with several indigent traits, which may promote the initiation of schizophrenia due to the presence of latent toxoplasmosis in their blood. Studies have shown that the peak onset of toxoplasmosis and schizophrenia are similar and occurs between the ages of 20 and 30 years [5,9]. A study in China reported that having antibodies to *T. gondii* at the time students enter college made it significantly more likely that the students would be diagnosed with schizophrenia during the next four years [9].

There is no trend or pattern observed in the relationship between the age groups of subjects and the seroprevalence of toxoplasmosis among the four sub groups. This same observation was reported in individual and comparative studies on toxoplasmosis where no relationship was found between age groups and seropositivity to toxoplasmosis [8,9]. However, some scholars observed that the seroprevalence of *Toxoplasma Spp* infection increases with age. This was attributed to the declining immunity and gradual onset of ageing [3,5,20].

The unemployed recorded the highest seropositivity to toxoplasmosis in this study which was 27.8% IgG. This may be attributed to

high stress levels and poor financial strength. Factors such as high stress levels and lack of finance may trigger behavioural changes that may lead to schizophrenia for which toxoplasmosis has been implicated [4,20]. Traders recorded the highest seroprevalence among HIV subjects (17.2%) for IgG. This may be due to the fact that more traders were examined in the study population.

There is however no trend or pattern as regards occupation and seropositivity to *T. gondii*. This has been reported by some other authors [4,13,14].

There was no visible relationship established between sex and seroprevalence. This observation was also made in several similar studies which reported no significance between sex and seroprevalence of toxoplasmosis did exist [13,14,23]. However more male schizophrenics and male HIV subjects were seropositive to toxoplasmosis than their female counterpart. However, some scholars in similar studies observed that males are three times more susceptible to toxoplasmosis than females [4,13,23].

From similar studies which border on risk factors which influence toxoplasmosis, it has been observed that poor personal hygiene greatly contributes to toxoplasmosis, provided environmental distinctiveness is not taken into consideration [4,13,23,24]. Observation made in this study concurs with these reports as drinking untreated water and eating improperly or unwashed fruits appear to greatly influence the seroprevalence of *Toxoplasma Spp*. IgG antibodies. This makes these factors obvious risk factor and great determinant of seroprevalence.

[25] strengthened the evidence that *Toxoplasma* Spp. infection may result from consumption of unwashed raw vegetables and fruits. They indicated that even though the percentage of vegetables and fruits contaminated with *Toxoplasma* Spp. may be small, the frequency with which these foods are consumed may result in appreciable exposure. Altogether these risk factors are consistent with the majority of infections being food borne [2,13,24].

Other factors which entail a history of owing with pets and eating undercooked suya were observed to have no significance in the present study. This finding is at variance with observations made by some scholars in similar studies where they implicated the possession of pets as one of the risk factors to transmission of toxoplasmosis [3,13,24]. However, statistical analysis shows no significant relationship between risk factors and seropositivity to toxoplasmosis.

4. CONCLUSION

The study showed that toxoplasmosis was significantly prevalent ($P=0.05$) among the studied populations with the schizophrenias having the highest seroprevalence. Drinking untreated water and eating improperly or unwashed fruits and vegetables were the risk factor majorly influencing the transmission of the parasite in this study.

It is recommended that health education; improved hygiene and routine tests should be adopted in the control of the disease.

CONSENT

According to laid down international standards written informed consent was obtained from the patient (or other approved parties) for publication of this study.

Written informed consent was obtained from all study participants and well-structured questionnaires capturing information regarding age, gender, occupation, possession of pets, drinking untreated water, engagement in farming, eating improperly or unwashed fruits and vegetables and eating partially cooked meat (suya) were administered to respondents.

ETHICAL APPROVAL

Ethical approval was sought and obtained from the appropriate ethics committee.

All tests were performed in accordance with laid down standards.

Ethical clearance was sought and obtained from the ethical committees of the University of Port Harcourt Teaching Hospital and Rivers State Hospital Management Board.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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