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Research Article

Serological and Molecular Prevalence of *Toxoplasma gondii* among HIV-infected Pregnant Women in Calabar, Nigeria

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ABSTRACT

Toxoplasma gondii infection (Toxoplasmosis) is a zoonotic parasitic infection that affects about 60% of the human population globally causing congenital, ocular, and cerebral infections in HIV-infected and other immunocompromised people. Morbidity and mortality data on toxoplasmosis as a congenital or as an opportunistic infection, especially among the HIV-infected subjects are lacking in the study area. The serological and molecular prevalence of *toxoplasmosis* in HIV-infected pregnant women in Calabar, Nigeria was investigated using a descriptive cross-sectional study. Blood samples randomly collected from one hundred and eighty (180) HIV infected and forty-five (45) non-HIV infected pregnant women, aged 10-50 years, were tested for anti-IgM and anti-IgG antibodies based on Enzyme Linked Immunosorbent Assay (ELISA) technique. Fifty samples positive for both IgM and IgG antibodies were selected and amplified by Polymerase Chain Reaction (PCR). The molecular and serological prevalence of toxoplasmosis were 50% and 52.8% respectively. There was no statistically significant association between *Toxoplasma* seropositivity and HIV status as seroprevalence of *T. gondii* among HIV infected pregnant women and their non-HIV infected pregnant counterpart were similar 52.8% vs 53.3%, respectively (P= 0.998). The highest seroprevalence, of 100% was recorded in those aged 50 years and above, while the least was seen in those aged 10-19 years, indicating seroprevalence to be age related (P<0.001). Demographic factors such as residential area, marital status, level of education and occupation significantly influenced the acquisition of *Toxoplasma* infections (P< 0.001). Keeping of pet animals and consumption of raw meat had significant effect on the acquisition of toxoplasmosis (P <0.05), whereas knowledge of the disease and source of drinking water had no significant effect (P >0.05). Age did not affect PCR amplification of Tox G1 gene (P >0.05). This study has confirmed high prevalence of toxoplasmosis among HIV and non-HIV infected pregnant women alike in Calabar. Intensive health education, routine diagnosis using ELISA antigen/antibody technique/prophylactic treatment for toxoplasmosis in all pregnant women and screening of new-borns for the true burden of congenital toxoplasmosis and a break in the zoonotic transmission cycle are recommended.

Keywords: *Toxoplasmosis, Pregnancy, HIV, Molecular-prevalence, Seroprevalence*

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INTRODUCTION

Toxoplasmosis, caused by Toxoplasma gondii, is one of the world's most common parasitic infections, infecting most genera of warm-blooded animals. It is the most prevalent infection in humans involving about 50 per cent percent of the world population (Torgerson and Mastroiacovo, 2013) causing congenital, ocular, and cerebral infections in HIV-infected and other immunocompromised people (Bahia-Oliveira *et al*., 2018).

Felines are definitive hosts while non-feline vertebrates, including humans, act as intermediate hosts of the parasite (Sudan *et al*., 2013). Humans become infected by contact with oocyst from cat's faeces or consumption of parasite in improperly cooked meat (Dubey, 1998)4. Seroprevalence survey among pregnant women globally varies from 7% to 51.3% (Dubey and Beattie, 1998)5 with a 30 percent chance of congenital infection (Ho-Yen and Joss, 1992).

In Nigeria, great variation in the prevalence of toxoplasmosis is documented among pregnant women in different localities e.g 43.7% in the Benue basin (Olusi *et al*. (1996)7, 40.4% in Zaria (Ishaku *et al*., 2009)8. Also, prevalences of 40.2% and 48.9% were reported for Lagos and Maiduguri, respectively (Deji-Agboola *et al*., 2011; Nasir *et al*., 2015).

Toxoplasmosis is generally a benign infection except when the disease occurs in pregnant women (congenital toxoplasmosis) or in immunocompromised individuals, such as immunodeficiency virus-positive or grafted patients, in which cases, the vital prognosis may be involved. Primary infections with *T. gondii* during pregnancy are usually asymptomatic but when infection is transmitted to the foetus, it may lead to miscarriage, hydrocephalus, cerebral calcification, choriorenitis, mental retardation, blindness, epilepsy and death of the new born (Satti *et al*., 2011; Flatt and Shatty, 2013)11, 12. The severity of the infection outcomes is dependent on the gestational stage of the pregnancy when the infection occurred (Montoya and Liesenfeld, 2004; Paquet *et al*., 2013)13, 14. A late manifestation of congenital or acute infection can also lead to toxoplasmic chorioretinitis in children and young adults (Montoya and Liesenfeld, 2004).

There are several serological tests available for the detection of T. gondii antibodies but recently, enzyme linked immunosorbent assays (ELISA) have gained a lot of utility in the diagnosis of toxoplasmosis (Sudan *et al*., 2013). Serodiagnosis is usually achieved by detecting IgG and IgM antibodies against T. gondii. The diagnostic challenges however, are difficulties in differentiating between primary and chronic infection and interpreting IgG and IgM results (Paquet *et al*., 2013). Where serological assays are unreliable or when the clinical diagnosis is doubted, molecular methods based on polymerase chain reaction (PCR) can be performed (Bastein, 2002). These techniques are simple, sensitive, and reproducible and can be applied to all clinical samples (Ivovic *et al*., 2012).

Toxoplasmosis has attracted renewed research interest because of its involvement in the pathogenesis of AIDS and other immunosuppressive diseases. Despite the recognized dangerous public and reproductive health effects of T. gondii in different parts of the world, data on its morbidity and mortality in congenital infection among pregnant women living with HIV/AIDS are scanty in Nigeria and unavailable in the study area. The aim of this study was to establish the molecular and serological prevalence of T. gondii infection among HIV-infected pregnant women in Calabar to improve their medical care. It is important to note that T. gondii infection is not routinely requested by attending physicians in Nigeria even among subjects with immune-suppression for laboratory confirmation.

MATERIALS AND METHODS

Study Area: The study was conducted in Calabar, the capital city of Cross River State, Nigeria. Calabar metropolis is made up of two local government areas, Calabar Municipality and Calabar South which lies within the rainforest belt of Nigeria. According to 2006 National Population Census, the population of Calabar is estimated at 371,022 (Ottong *et al*., 2010)[17]. Geographically, Calabar is located on latitude $04^{o}.57^{ft}$ west and longitude $08^{o}.20^{ft}$ east with an area of 406 square kilometers. It is a large urban city with several hotels, good road network, hospitals, schools, many churches and other establishments. Most of the residents of Calabar are Civil Servants with only a small percentage engaged in farming and trading. The annual Calabar carnival attracts thousands of tourists beyond Nigeria into the city. The sample sites are the major government reference hospitals in the city, the University of Calabar Teaching Hospital and the State-owned General Hospital, Calabar.

Study Subjects: The study subjects comprised of pregnant women living with HIV and attending ante natal clinics in University of Calabar Teaching Hospital and General Hospital in Calabar and HIV negative pregnant women attending clinics at the time of study.

Study Design: A cross-sectional study was conducted in a span of twelve (12) months.

Administration of Questionnaires: Interview using structured and pretested questionnaires were performed to investigate risk factors associated with *T. gondii* infection, including consumption of raw meat and keeping pets (cats and dogs), and to collect data on sociodemographic characteristics of study subjects.

Inclusion and Exclusion Criteria: Only HIV positive pregnant and HIV negative pregnant women attending clinics at the time of study who gave their consent were included in the study.Those who refused to give their consent were excluded from the study.

Collection and Handling of Samples: Random sampling technique was used to select study participants. Samples were collected from patients who attended ante natal clinics and were referred to HIV Screening clinics by Medical Laboratory Scientists attached to the clinics.

About 5 ml of venous blood was collected aseptically from each of the 180 confirmed HIV positive pregnant women as well as from 45 HIV negative pregnant subjects into plain vacutainer tubes and EDTA bottles. The samples were transported to the Microbiology Laboratory in the University of Calabar Teaching Hospital (UCTH) where serum was separated from the clotted whole blood by centrifugation at 3,000 rpm for 5 minutes. Separated sera were then numbered and kept in the refrigerator at −20°C until they were used. Buffy coat were also separated from EDTA samples into a sterile screw capped cryovial, labelled and stored in the refrigerator at -20°C until required.

Screening for HIV: A Parallel Testing algorithm was employed, and was performed using HIV 1/2 STAT-PAK ASSAY (Chembio Diagnostic systems Inc. USA, batch No. HIV101N) and Uni-Gold HIV (Trinity Biotech, Ireland, batch No. 1206502N-100) kits according to manufacturers' instructions.

Serological Screening for Toxoplasmosis: Sera were tested for anti-*T. gondii* antibodies using BioCheck Toxoplasma ELISA test kits (BioCheck, Inc 425 Eccles Avenue, South San Francisco, CA 94080) for anti-*T. gondii*-specific IgG and IgM. The test was carried out according to the manufacturer's instructions.

Molecular Detection of *T. gondii*: Fifty (50) samples seropositive for both anti-*T. gondii* IgM and IgG were randomly chosen for molecular analyses.

DNA Extraction: DNA was extracted from the collected EDTA blood samples by chemical (kits) method using a commercial purification system (Quick-gDNA MiniPrep by Zymo Research Corporation) following the manufacturer's instructions.

Quantification of DNA: Prior to PCR analysis, the eluted DNA was quantified by Nano dropping using Nano Drop 1000 spectrophotometer and the concentration in ng/µl was read at absorbance of 260/280.

Polymerase Chain Reactions (PCR) Assay: PCR assay was performed on all DNA samples to amplify a fragment from the Tox G 1 genes conserved in the *T. gondii* genome. The assay was performed following the protocol described briefly.

Amplification of Tox G1 gene of *Toxoplasma gondii:* Tox G1 gene from the samples was amplified using the Tox: (forward primer) 5' CGCTGCACGGAGGAAGACGAAAG TTG 3' and Tox5: (reverse primer) 5' CGCTGCACACACA GTGCATCTGGATT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master Mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 58ºC for 40 seconds; extension, 72ºC for 50 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The PCR amplicons were resolved on 1.5% agarose gel electrophoresis tinted with E-Z vision dye and visualized on a blue light transilluminator.

Ethical Approval and Informed Consent: Ethical clearance was sought and obtained from Cross River State Committee on Research Ethics and informed consent was obtained from the patients or their guardians prior to their enrolment in the study.

Data Analysis

The data generated in this study were analysed using the PASW (SPSS) version 25.0. The Frequency and Mean of the experimental variables were obtained. Chi square Test, was also used where applicable, and was assessed at a statistical significance level of ($P < 0.05$).

RESULTS

The socio-demographic characteristics of the study participants and their gross ELISA reaction to *T. gondii* are shown in Table 1. Of the 180 pregnant subjects, the highest number of participants 93(51.6%) was recorded in those aged 30-39 years followed by those aged 20-29 years with 71(39.4%) participants, age group 10-19 years, 8(4.4%), age group 40-49 years, 6(3.3%) and 2(1.1%) from those aged 50 years and above, Overall, 95 (52.8%) of the 180 HIV positive pregnant women were toxoplasmosis seropositive with ELISA method with the highest seropositivity 2 (100%) obtained from those aged 50 years and above, closely followed by age group 40-49 years, 4 (66.7%), while the lowest seropositivity, 3 (37.5%) was recorded in the 10-19 years age bracket. There was an association between age and *T. gondii* seropositivity (X^2 =34.039, P<0.001).

One hundred and sixty-six (92.2%) urban dwellers took part in the study, while 14 (7.8%) of the participants came from rural areas. The seropositivity level for *T. gondii* was higher in urban 136(81.9%) than in rural residents 3 (21.4%). This difference was statistically significant (X^2 =41.078, P<0.001). On level of educational attainment, 20(11.1%) of the participants had no formal education as against 38(21.1%) who attained primary level, while 90(50.0%) and 32(17.8%) attained secondary and tertiary levels, respectively.

Table 1:
Socio-demographic characteristics of HIV pregnant women and their gross ELISA reactions to *T. gondii*

	Sub-variable	No (%) Examined	No (%) positive for *T. gondii*	Chi square test
Age (Years)	10-19	8(4.4)	3(37.5)	
	20-29	71(39.4)	41(57.7)	Chi cal. = 34.039
	30-39	93(51.6)	45(48.4)	df = 4; p<0.001
	40-49	6(3.3)	4(66.7)	
	50 & above	2(1.1)	2(100)	
	Total	**180**	**95(52.8)**	
Residential Area	Urban	166(92.2)	136(81.9)	Chi cal. = 41.078
	Rural	14(7.8)	3(21.4)	df = 1; p<0.001
Level of Education	Non-formal	20(11.1)	15(75.0)	
	Primary	38(21.1)	21(55.2)	Chi cal. = 23.269
	Secondary	90(50.0)	79(87.8)	df = 3; p<0.001
	Tertiary	32(17.8)	24(75.0)	
Occupation	Civil servants	21(11.7)	19(90.5)	
	Artisans	37(20.5)	28(75.7)	Chi cal. = 24.577
	Students	39(21.7)	22(56.4)	df = 3; p<0.001
	Unemployed	83(46.1)	71(85.5)	

Table 2:
Non-HIV pregnant women (controls) and their gross ELISA reactions to *T. gondii* based on age

Age (Years)	No (%) Examined	No (%) positive for *T. gond*
10-19	3 (6.7)	1 (33.3)
20-29	19 (42.2)	9 (47.3)
30-39	9 (20.0)	4 (44.4)
40-49	10 (22.2)	6 (60.0)
≥ 50	4 (8.8)	4 (100)
Total	45 (100)	24 (53.3)

Table 3:
Participants' Knowledge, Predisposing Factors and their Gross Serological reaction to Toxoplasmosis

Variable	Sub-variable	No (%) Examined (n=280)	No (%) with Toxoplasmosis
Knowledge of Toxoplasmosis	Yes	5(2.7)	3(60.0)
	No	175(97.2)	137(78.3)
	Chi cal. = 1.706; df = 1; p = 0.191		
Source of Drinking Water	Borehole	124(68.9)	93(75.0)
	Pipe borne	55(30.5)	45(81.8)
	River	1(0.6)	1(100)
	Chi cal. =1.6207; df = 2; p = 0.445		
Keeping of Pet Animals	Cats	45(25.0)	41(91.1)
	Dogs	38(21.1)	19(50.0)
	None	97(53.9)	81(83.5)
	*Chi cal. = 30.250; df = 2; p = 0.01		
Consumption of Raw Meat	Yes	21(11.6)	21(63.6)
	No	159(88.3)	127(79.9)
		*Chi cal. = 4.123; df = 1; p =0.042	

**=Keeping of Pet Animal and Consumption of Raw Meat significantly affected Occurrence of Toxoplasmosis.*

Seroprevalence of *T. gondii* was higher 79(87.8%) among those who attained secondary level of education than those with no formal education 15(75.0%) and tertiary level 24 (75.0%). The lowest prevalence in the group, 21(55.2%), was found in those with primary level of education. There was association between *T. gondii* seropositivity and level of educational of subjects (X^2 =23.269; P<0.001). Unemployed people 83(46.1%) formed the bulk of participants in the occupational status group followed by students 39(21.7%), artisans 37(20.5%) and civil servants 21(11.7%). The seroprevalence was highest in civil servants 19(90.5%), followed by unemployed 71(85.5%), artisans 28(75.7%) and lowest in students 22(56.4%). The rate of detection of *T. gondii* antibodies based on occupation was statistically significant (X^2 =24.577; P<0.001).

Table 2 is on the non-HIV pregnant women (controls) and their gross ELISA reactions to *T. gondii* based on age of subjects. Of the 45 non-HIV pregnant (control) subjects screened for gross ELISA reaction, 24 (53.3%) were toxoplasmosis seropositive. Age group 50 years and above showed the highest seropositivity, 4 (100%) while age group 10-19 years had the least, 1 (33.3%).

Table 3 shows the participants' knowledge, predisposing factors and their gross serological reaction to toxoplasmosis. Out of the 180 subjects who participated in the study, only 5(2.7%) affirmed to have knowledge of toxoplasmosis while 175(97.2%) had no knowledge of the infection. There was a higher prevalence 137(78.3%) in those who had no knowledge of the infection than in those with the knowledge 3(60.0%). However, knowledge of the disease does not have any significant effect on the infection (X^2 = 1.706, P = 0.191). The source of drinking water of the study subjects was also considered. Majority of them 124(68.9%) used borehole water as their drinking source, while 55(30.5%) used municipal water supply (Pipe borne water) and 1 (0.6%) drink water from the river. Source of drinking water and the acquisition of toxoplasmosis were not statistically related (X^2 = 1.6207, P= 0.445), although seroprevalence was higher in those who used river water 1(100%) than those who use pipe borne water 45(81.8%) and borehole 93(75.0%) as their sources of drinking water.

The association between keeping of pet animals and infection with *T. gondii* was also studied. A higher percentage of the test subjects 97(53.9%) did not keep pet animals, while 45(25.0%) preferred cats to dogs 38(21.1). Seroprevalence was highest in those who keep cats 41(91.1%), followed by those who do not keep pets 81(83.5%) and those who keep dogs 19(50.0%). Keeping of pet animals had significant effect on the acquisition of toxoplasmosis (X^2 = 30.250, P = 0.001). On consumption of raw meat, 21(11.6%) of the study subjects liked to eat their meat raw while 159(88.3%) did not eat raw meat. Seroprevalence of toxoplasmosis was higher in those who do not eat raw meat 127(79.9%) than in those who eat 21 (63.6%). There was a relationship between eating of raw meat and *Toxoplasma* infection (X^2 = 4.123, P = 0.042).

Table 4:
Detection of *T. gondii* IgM and IgG Antibodies among 95 gross seropositive test subjects by Age

Age group (years)	No (%) Examined	Gross (%) Abs detection	IgG	IgM	IgG/IgM
10-19	8(4.4)	3(37.5)	3(37.5)	3(37.5)	3(37.5)
20-29	71(39.4)	41(57.7)	41(57.7)	41(57.7)	41(57.7)
30-39	93(51.6)	45(48.4)	45(48.4)	45(48.4)	45(48.4)
40-49	6(3.3)	4(66.7)	4(66.7)	4(66.7)	4(66.7)
≥ 50	2(1.1)	2(100)	2(100)	2(100)	2(100)
Total	180(100)	95(52.8)	95(52.8)	95(52.8)	95(52.8)

Key: *Abs = Antibodies*

The Agarose gel electrophoresis of the amplified Tox g1 gene of the Toxoplasma gondii is shown in Plate 1. The positive DNA band of conventional PCR reactions showed 550 base pairs in length. Lanes 1, 3-5, 7-9 and 11 represent the Tox g1 gene at 550bp. Lane L represents the 100bp molecular ladder, while lanes 2, 6 and 10 represent absence of Tox g1 at 550bp.

Table 4 displays the detection of *T. gondii* IgM and IgG Antibodies among 95 gross seropositive test subjects by Age. All of the 95 *T. gondii* gross seropositive test subjects were detected with both IgG and IgM antibodies.

Table 5 shows the PCR results obtained from the amplification of *T. gondii* Tox G1 gene by age of randomly selected 50 subject's samples positive for both IgM and IgG *T. gondii* antibodies. The overall result shows that 25 (50%) of the samples were PCR positive. Age however, did not influence PCR result ($X^2 = 3.143$, $P = 0.5342$).

Table 5:
PCR Results Obtained from Amplification of *T. gondii Tox G1* Gene by Age

Age group (in years)	No (%) Examined	No (%) Positive
10-19	2 (4.0)	1 (50.0)
20-29	9 (18.0)	6 (66.7)
30-39	22 (44.0)	10 (45.4)
40-49	13 (26.0)	6 (46.1)
≥ 50	4 (8.0)	2 (50.0)
Total	50 (100)	25 (50.0)

DISCUSSION

The seroprevalence of *T. gondii* infection in this study was 52.8% for IgG, IgM and IgG/ IgM antibodies, while the molecular detection showed a prevalence of 50%. The overall seroprevalence of *T. gondii* in this study agrees with the global estimates which are put between 1% and 100% (Furtado *et al.*, 2011). However, it varies significantly with 6.7% reported in Korea (Shin *et al.*, 2009), 12.3% in China (Xiao *et al.*, 2010), 22.5% in USA (Jones *et al.*, 2001), 42.0% in Iran (Rahmanian *et al*., 2020), 28.3% in Thailand (Nissapatorn *et al.*, 2011), 23.9% in some regions in Nigeria (Kamani *et al.*, 2009) and 46% in Tanzania (Swai and Schoonman, 2009). It is lower than 67% in Brazil, 68% in Germany and much lower than 80% in the Netherlands (Henriquez *et al.*, 2009) and 98% in some regions of France (Silveira *et al.*, 1988). The reason for this variation in prevalence worldwide is not clearly understood, but as suggested by Furtado *et al.* (2011), this could be as a result of the differences in environmental and socioeconomic conditions, including eating habits and health-related practices, general level of hygiene, host susceptibility, geographical location (geolatitude) and humidity of the soil.

Among HIV/AIDS patients, seroprevalence of 52.8% for both IgG and IgM in the present study disagrees with other works done by Rodier *et al.* (1995) on HIV infected women in Benin Republic where prevalence of 28.5% was reported and 40.2% reported in Senegal by Assob *et al.* (2011) who worked on Toxoplasma antibodies amongst HIV/AIDS patients attending the University Teaching Hospital, Yaoundé, in Cameroon. It however agrees with the work of Pappoe *et al.* (2017) in a study of prevalence of *T. gondii* infection in HIV infected patients and food animals and direct genotyping of *T. gondii* isolates in Southern Ghana which reported a prevalence of 74.4% from both IgG and IgM antibodies. The reason for this difference may be attributed to different study population and laboratory procedures.

This present study found that seroprevalence in pregnant women measured by both IgG and IgM was 52.8% (see Table 4). This was higher than 40% by Rahmanian *et al.* (2020) in Iran, 36.3% in Thailand (Chemoh *et al.*, 2015) and 37.5% reported by Okwuzu *et al.* (2014) in Lagos, Nigeria but lower than 71% observed in Ethiopia (Feleke *et al.*, 2019). Similar studies conducted in Nigeria includes that of Yusuf *et al.* (2016) on HIV infected pregnant women in Kano with 34% prevalence, 39% among pregnant women in Kaduna (Aganga *et al.* (1990), 22% in pregnant women in Borno (Oyinloye *et al*., 2014) and 41% in Lagos (Akinbami *et al.* (2010). The variation in the prevalence may be attributed to the different locations where the studies were conducted and the different methodologies used for serology. This is the first study in Nigeria that report on the prevalence of *T. gondii* antibodies among HIV infected pregnant women in the South-southern part of the country.

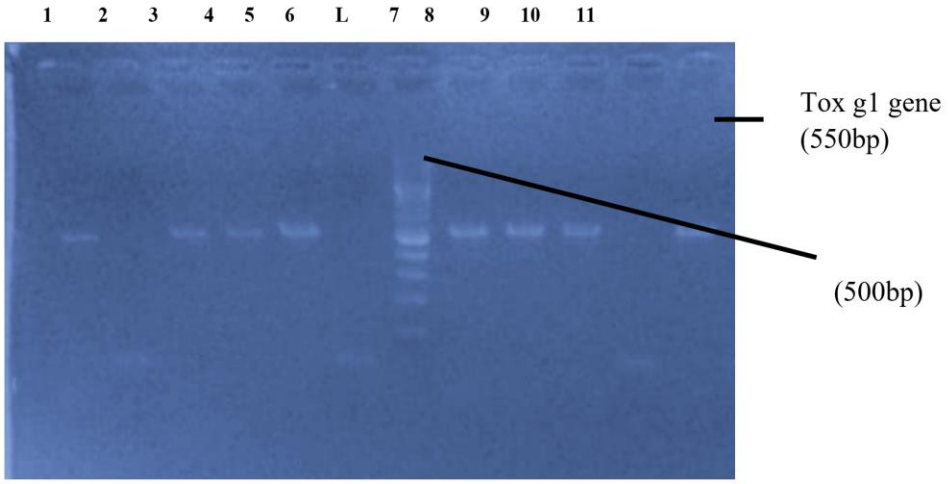

Plate 1:
Agarose gel electrophoresis showing the amplified Tox g1 gene of the *Toxoplasma gondii.* Lanes 1, 3-5, 7-9 and 11 represent the Tox g1 gene at 550bp. Lane L represents the 100bp molecular ladder. Lanes 2, 6 and 10 indicate absence of Tox g1 gene.

The overall seroprevalence of *Toxoplasma* infection measured by specific anti *Toxoplasma* IgG in this study was 52.8%. This finding agrees with the work of Ekanem *et al.* (2018) who reported a prevalence of 55.8% measured by IgG in Uyo, Nigeria. The prevalence is higher than 25.3% recorded in Burkina Faso by Jacques *et al.* (2006), 33.5% in Iran (Mousavi-Hasenzadeh *et al.,* 2020), 28.3% in Thailand (Nissapartorn *et al.,* 2011) and 9.3% by Findel (2015) in Norway but lower than 75.4% in Oyo, Nigeria (Onadeko *et al.,* 1995), 74.4% (Pappoe *et al* (2017) in southern Ghana, 81% and 88.5% by Feleke *et al.* (2019) in HIV positive individuals while conducting a systemic review and meta-analysis on toxoplasmosis in pregnant women and HIV patients in Ethiopia. The overall high prevalence reported in this study agrees with Robert-Gangneux and Darde, (2012) who noticed high prevalence in Latin America and tropical Africa.

No documented prevalence of Toxoplasmosis on HIV-infected pregnant women was found in the South- south region of the country. The variation in the prevalence of *Toxoplasma* infections among pregnant women living with HIV recorded across the country may be attributed to the different sample sizes and different serological methods employed by the different researchers.

In this study, it was found that keeping of pet animals had significant effect on *T. gondii* infection (X^2 = 30.250, P< 0.05). Seroprevalence was higher in those who keep cats than those that keep dogs as pets (Table 3). This finding is in line with that of Okwuzu *et al.* (2014) who observed that living in close proximity with cats was significantly associated with *T. gondii* infection, but contradicts that of Muluye *et al.* (2013) in Ethiopia, who found no association between keeping pet animals and *T. gondii* infection. Some studies have reported that seropositivity of *T. gondii* is associated with eating of rodents, having soil related occupation, cat ownership (Uneke *et al.,* 2005). The association of *T. gondii* infection with cats in the present study can be justified because in our environment, as noted elsewhere (Okwuzu *et al.,* 2014) and depicted in Table 3, most people who were seropositive, 97 (53.9%) in this study do not own cats but may have acquired the infection from cats that gained access to their residences especially in the night where they look for leftover food leaving their droppings to contaminate the surfaces.

The findings of this present study indicated that age significantly influenced the acquisition of *Toxoplasma* infection (X^2 = 66.940, P = 0.000). Higher levels of *T. gondii* antibodies were found in those aged 40 years through 50 years and above. This finding is in conformity with that of Zhang *et al* (2015) who reported rising antibody levels in 3rd and 4th decades of life in China. Previous studies in India (Sarkar *et al.,* 2012) and Ethiopia (Zemene *et al.,* 2012) also showed that seropositivity of *T. gondii* infection increases with age. This can probably be explained by the prolonged exposure time with the increase of patient age. On the contrary however, the result of this study differs from that Walle *et al.* (2013) in Ethiopia and Okwuzu *et al.* (2014) in Nigeria who reported highest prevalence in age group 21-30 years. The reason for this difference is not clear, but may be attributed to the different categories of study participants used.

The high molecular prevalence of *T. gondii,* 50% in this study is close to the 57.9% recorded in Libya by Gashout *et al.* (2016) but higher than 41% reported in Saudi Arabia among pregnant women (Dajem and Almushait, 2012) and significantly higher than 17.9% reported in Scotland by Burrells *et al.* (2016), 11.8% in Egypt (Ibrahim *et al.* 2017), 11.6% in central Iran (Bahreh *et al.,* 2021) and 26% in Iran (Anvari *et al.,* 2018).

The reason for the difference in molecular prevalence is not clearly understood, but may be as a result of different level of hygienic practices and awareness of preventive measures in different parts of the world.

In the current study, age did not influence PCR result (P = 0.534). This disagrees with the report of Ibrahim *et al.* (2017) who observed that positivity was higher in those who are 25 years and above than younger people and Burells *et al.* (2016) who noticed a direct link between increasing positivity and increasing age.

In conclusion, we present a first serological and molecular prevalence data, 52.8% and 50%, respectively for human *T. gondii* infection among HIV/AIDS pregnant women in the study area. This prevalence is high and is attributed to keeping of pet animals like cats, 91.1% and dogs 50.0%, as well as consumption of raw or undercooked meat and vegetables (63.6%). There was no association between *Toxoplasma* seropositivity and HIV status but age, residential area, marital status, level of educational attainment and occupation were found to be risks factors in acquiring toxoplasmosis.

The high prevalence shows a great tendency for toxoplasmosis-HIV related complications in such people. To prevent these avoidable complications, intensive health education, and a break in the animal/man link in the disease transmission cycle (including prevention of stray cats and dogs from entering homes, adequate cooking of meat and proper food hygiene) are recommended. Highly efficient and cost-effective antigen/antibody detection ELISA assay is also recommended for routine screening with early treatment for toxoplasmosis in all HIV- positive (and other) pregnant women. and new-borns over a specified time period to elucidate the true burden of congenital toxoplasmosis.

Many patients, especially males, refused to give their consent, hence the greater number of females than males subjects. Furthermore, there was no other means of ascertaining that the responses by the study participants concerning their sociodemographic characteristics were all correct. Storage of samples at the required temperature of -20^{0}C was a big challenge as there was hardly any refrigerator that could maintain exuberated by epileptic power supply. Due to limited funds, strain genotyping by PCR-Restricted Fragment Length Polymorphism (PCR-RFLP) was not performed on the *T. gondii* isolates.

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