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Toxoplasma gondii infection: seroprevalence and associated risk factors for women of childbearing age in Osun State, Nigeria

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ABSTRACT

Toxoplasmosis is a common parasitic infection caused by an obligate intracellular protozoan, *Toxoplasma gondii*. Prevalence and risk factors of *T. gondii* infection in women of childbearing age in Osun State, Nigeria are unknown. This study was aimed to determine the seroprevalence and potential risk factors in acquiring *T. gondii* infection by women of childbearing age in Osun State, Nigeria. A community-based cross-sectional study was conducted from May 2019 to December 2019 in childbearing age women. Sera of 415 women aged 18–49 years randomly selected were collected and analyzed by enzyme-linked immunosorbent assay (ELISA) test. A questionnaire survey was administered for all study participants to collect socio-demographic and risk factors data. The study revealed that the overall seroprevalence of *T. gondii* infection was 76.63%, which comprised 6.02% positivity for anti-*T. gondii* IgM (25/415), 44.10% for IgG (183/415) and 26.51% for IgG plus IgM (110/415). Seroprevalence of IgM antibodies to *T. gondii* (6.02%) suggested recent infections. Women residing in rural communities and women of Islam religion showed significant association with anti-*T. gondii* seropositivity ($p < 0.05$). Residence location and women who are of Islam religion are risk factors to acquire *T. gondii* infection. Hence, health education and awareness on the disease and its transmission to women of childbearing age group in general and pregnant women in particular should be created during antenatal follow up to reduce the risk of *T. gondii* infection in pregnant women.

KEYWORDS

Toxoplasma gondii; seroprevalence; ELISA; risk factors; women of childbearing age; Osun State; Nigeria

Introduction

Toxoplasma gondii is an obligate intracellular protozoan that is globally widespread and causes a common infection (known as toxoplasmosis) in animal and human. *T. gondii* uses felines as its definitive host including domestic cats, which are the main source of infection through oocysts passed in their feces. Almost all warm-blooded mammals, including livestock and human can serve as intermediate hosts [1]. *T. gondii* infection represents the most prevalent parasitic zoonotic disease [2,3]. It has been estimated that nearly one-third of the world's population is infected with *T. gondii* [4].

Human infection may occur via several routes including close contact with infected cats or soil, consumption of raw or uncooked meat infected by cysts of *T. gondii*, ingestion of food or water contaminated with oocysts excreted in the feces of cats, blood transfusion or organ transplants, intrauterine or transplacental transmission and drinking infected unpasteurized milk [5]. *T. gondii* infection acquired during pregnancy can be transmitted to the fetus sometimes with serious consequences [6]. It can result in fetal death, neonatal death or various congenital defects, such as hydrocephalus, central nervous system abnormalities, premature birth, intrauterine growth retardation, fever, hepatosplenomegaly or involve eyes and brain [7]. Manifestation of ocular or encephalic disease in the fetus may include chorioretinitis, meningoencephalitis and microcephaly [7]. When women acquire *T. gondii* infection more than 6 months

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prior to gestation, risk of transmission to the fetus is considerably reduced. However, preventable and treatable congenital, ocular and postnatal *T. gondii* infection is not curable and persist in all infected persons [5,7,8].

Serological screening for *T. gondii* antibodies should be conducted in women of childbearing age as it allows identification of women at risk of acquiring infection [9] and is part of a strategic approach for prevention of congenital toxoplasmosis [10]. *T. gondii* antibodies are indicative of infection, and that infection is long lasting (generally thought to last throughout life). IgM anti-*T. gondii* antibodies are usually known as a marker of acute infection that appears earlier and decreases faster than IgG antibodies. IgM antibody is frequently first to be detected after the primary infection [11]. However, *T. gondii*- specific IgM can sometimes persist for up to 18 months after infection, giving rise to false-positive diagnoses of acute infection when no additional tests for *T. gondii*-specific IgG were conducted [9,11]. Thus, the diagnosis of recently acquired *T. gondii* infection is generally based on the detection of specific IgM antibodies, followed by detecting the specific IgG antibodies 1–3 weeks later [11].

The seroprevalence of *T. gondii* infection among women of childbearing age in different countries ranges from 4 to 100% [2,9]. In Africa, seroprevalence ranges from 25% in Burkina Faso to 81.4% in Ethiopia [12,13]. In Nigeria, toxoplasmosis is a neglected disease and infection in women of child bearing age is unknown. From the studies conducted in Nigeria, a prevalence ranges from 2.00% to 88.24% was reported in Nigerian population [14]. Pooled seroprevalence of *T. gondii* reported in Nigeria among subgroups include 40.25% in pregnant women, 23.32% in healthy individual, 36.93% in adults, 18.52% in children and 31.68% in HIV patients [14].

Due to the asymptomatic nature of *T. gondii* infection, the need for counseling of pregnant women to reduce the risk of fetal infection cannot be overemphasized. Hence, effective counseling for prevention requires the knowledge of the risk factors associated with the transmission of the parasite [15]. Therefore, this study was conducted to determine the seroprevalence and potential risk factors which facilitates the acquisition of *T. gondii* among women of childbearing age in Osum State, Nigeria.

Materials and methods

Study area

Osun State is situated in the tropical rain forest zone and covers a land area of approximately 14, 875 sq. km and lies between latitude 7° 30´N and longitude 4° 30 ´E. Osun state is bounded in the south by Ogun State, in the north by Kwara State, in the west by Oyo State and in the East by Ekiti and Ondo States. According to 2006 National Population Census figure, Osun State has a total population of 3,423,535. Osun State consists of 30 local government areas (LGAs). The communities selected for the study in Osun State include Alajue, Edunabon, Erin Ijesa, Ifewara, Ikire, Ile-Ife and Kajola. The communities, their LGAs, geographical coordinates and the population size are given below.

Communities	Local government area (LGA)	Coordinates		Population size
		Latitude	Longitude	
Alajue	Ede South	7° 42´N	4° 27´E	NA
Edunabon	Ife North	7° 33´N	4° 27´E	122,996[16]
Erin Ijesa	Oriade	7° 30´N and 8° 45´N	4° 31´E and 5°E	8,111[16]
Ifewara	Atakumosa West	7° 25´N and 7° 31´N	4° 32´E and 4° 35´E	76,197[16]
Ikire	Irewole	7° 21´N	4° 11´E	143,599[16]
Ile-Ife	Ife Central	7° 26´N and 7° 33´N	4° 35´E	188,000[16]
Kajola	Atakumosa West	7° 33´N	4° 37´E	NA

NA = not available.

The climate is typically tropical with a characteristic dry season of about 6 months (October–March) and a wet season of about 6 months (April–September). Annual rainfall ranges between 1000 and 4000 mm [17–18]. The average maximum and minimum temperatures are 32°C and 20°C, respectively [17]. The vegetation is rainforest characterized by large and tall trees.

The inhabitants of these communities are a mixture of people from different ethnic groups, although the majority are Yoruba-speaking people of the Southwest. Majority of the inhabitants are skilled workers, e.g. civil servants and artisans while others are unskilled workers, e.g. peasant farmers, traders and transport workers. These people share a close relationship with semidomesticated animals such as cats and dogs, often allowing them enter into their houses. Cats are seen in the communities roaming freely through the compounds of the residences to which they are affiliated.

Study population

The study population comprises women of childbearing age, aged 18–49 years who attended monthly clinic organized by a Non-governmental Organization, a mobile clinic, St. Andrew's Clinic for Children Ile-Ife (STACCILEIFE). The mobile clinic offers free medical services for children aged 0–5 years in 10 rural communities in Osun State where sick children are treated on various infectious diseases. During the clinic at each selected community, women who brought their children for medical attention were approached and the purpose of the study and

procedures to be adopted were explained to them. Women who were interested were asked to register their names and the consent form was given to them to either sign or thumbprint. Inclusion criteria for participation of the women were (i) women aged 18–49 years old, (ii) women that signed or thumbprint their consent forms and (iii) women resident in the selected communities.

Ethical consideration

The research protocol was submitted and approved by the Health Research Ethical Committee of the Institute of Public Health (IPHOAU/12/1318), Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. Prior to the commencement of the study, permission was sought from the various local government authorities. Meetings were held with the leaders of the community and the women to explain the objectives and protocol of the study.

Sample size determination

The sample size was calculated using the formula given below at 95% confidence interval (CI) level = 1.96, an expected prevalence of 50% (since no prevalence study has been conducted among women of childbearing age in Osun State) and 5% precision.

$N = Z$ [2]. p. (1-p)/ d [2]; where N = sample size, Z = confidence level = 1.96, p = expected prevalence = 0.5, d = precision = 0.05. The sample size was increased by 5% to account for attrition = 403, which is the minimum samples to be collected.

Study design

A community-based cross-sectional study was conducted from May 2019 to December, 2019. A total of 545 women of childbearing age who were eligible to participate in the study from the selected seven communities registered, out of which 415 who signed or thumbprint the consent forms were randomly selected. The women were instructed to register their names if they were interested in participating in the study. From the registered names, simple random sampling method was used to select women from each community using a table of random numbers and when the selected woman is absent, the woman before or after was sampled as a replacement.

Socio-demographic data including age, residence place, marital status, occupation, educational level and housing condition (house floor type) were collected using structured questionnaire. The questionnaire was also used to assess risk factors that include presence of cats at home, contact with cats, source of water drinking (tap, sachet, well, borehole, stream, well), consumption of raw/undercooked meat, hand washing after handling raw meat, consumption of raw vegetables, exposure to soil, and study participants' awareness about toxoplasmosis.

Sample collection and transportation

Blood samples of 5 ml were drawn from the selected women into plain, clean blood collection tubes by medical laboratory scientist. The blood samples were centrifuged at 3000 rpm for 5 min to obtain sera. Based on a Bilateral Research Cooperation Memorandum between Taipei Medical University and Nigerian Institute of Medical Research from 2018, the sera were collected into Eppendorf tubes and stored at −20°C until transported in an ice box to the Parasitology Laboratory of the Department of Molecular Parasitology and Tropical Diseases, TMU, Taiwan, where they were kept at −20°C until used.

ELISA analysis

Sera were analyzed for the presence of IgG and IgM antibodies against *T. gondii* using commercially available enzyme-linked immune assay kits and conducted according to the manufacturer's instructions (General Biologicals Corp., Hsin Chu, Taiwan). The performing procedure is described briefly. Add 100 µl of 1:40 diluted sera, calibrators, positive and negative controls into the appropriate wells. For the reagent blank, only 100 µl sample diluent was added into the appropriate wells; thereafter tap the holder to remove air bubbles from the liquid and mix well. The plate was then put into the incubator at 37°C for 30 min. Remove liquid from all wells and rinse and flick the microtiter wells five times with diluted wash buffer. Add 100 µl of enzyme conjugate to each well then mix gently for 10 s and then incubated at 37°C for 30 min. After washing five times with diluted wash buffer, add 100 µl of TMB reagent into each well and mix gently for 10 s and then incubated at 37°C for 30 min. Finally, add 100 µl of stop solution (1 N HCl) to stop reaction. Mix gently for 30 s to make sure that all the blue color changes to yellow color completely. Read O.D. at 450 nm within 15 min with a microwell reader. Calculate the *Toxoplasma* IgG or IgM Index by dividing the mean values of each sample by calibrator mean value. If Toxo G or IgM Index less than 0.90 indicates negative, while index between 0.91–0.99 is regarded borderline (weak positive), when the index 1.00 is regarded strong positive. The IgG test kit has a reported sensitivity and specificity of 98% and 99%, respectively, while the IgM test has a reported sensitivity and specificity of 100% and 97%, respectively [13].

Statistical analysis

The data were entered into Microsoft Excel spreadsheet (Microsoft Corporation,) and statistical analysis was conducted using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA). The seroprevalence was calculated as the number of serologically positive samples divided by the total number of samples tested expressed in percentage. Assessment of associations between demographic characteristics and *T. gondii* infection was performed by a Chi-squared test and used to compare proportions of infections based on gender, age group, religion, occupation, and educational level. The strength of the association was assessed by odds ratios and 95% confidence intervals (CI), which were calculated. Results were considered significant at $p < 0.05$.

Results

The demographic characteristic of women of childbearing age with respect to *T. gondii* infection in the selected seven communities in present study is described in Table 1. The participants' mean age ± SD was 27.95 ± 6.33 years old.

The overall seroprevalence of *T. gondii* among women of childbearing age was 76.63% (318/415), out of which 6.02% (25/415) were positive for anti-*T. gondii* IgM antibodies, 44.10% (183/415) positive for IgG and 26.51% (110/415) were positive for IgG plus IgM (Table 1).

As shown in Table 1, the seroprevalence *T. gondii* infection among women whose religion is Islam was significantly higher (88.81%) than those who are Christian (70.82%) ($p < 0.0001$). Another significant difference was observed in the seroprevalence of infection, which was significantly higher among women who had no formal or primary education (86.9%) than those with either secondary or tertiary education (74.0%) ($p < 0.01$). No significant differences were observed in univariate analyses with regard to age, location, occupation and house floor type (Table 1).

The risk factor analysis showed that the seroprevalence of women who had contact with cats (76.67%) and those who had no contact with cats (76.62%) were similar. The seroprevalence of women who drank unboiled water (76.32%) and those who did not (76.53%) were also similar. The risk factor analysis showed that there was no significant association between the risk factors and *T. gondii* seropositivity ($p > 0.05$). (Table 2).

Table 3 shows the result of the multivariate analysis. Multivariate logistic regression analysis showed that only study location ($p = 0.0001$) and religion ($p = 0.0001$) were associated with *T. gondii* seropositivity. Women of childbearing age who reside in rural community shows higher risk of contracting *T. gondii* infection than those who reside in peri-urban communities [adjusted odd ratio (AOR) = 0.39, CI: 0.23–0.69]. None of the risk factors (environmental factors) show a significant association with *T. gondii* infection including contact with cats, consumption of raw and frozen meat, drinking unboiled water, consumption of raw vegetable, handling raw meat and miscarriage (Table 3).

Table 1. Demographic characteristic of women of childbearing age with respect to *Toxoplasma gondii* in the selected seven communities in Osun State Nigeria.

Variables	Infection rate			
	N	%	OR	*p*-value
Age (median = 27)				
≤27 (*N* = 209)	156	74.64	1.00	0.33
>27 (*N* = 206)	162	78.64	1.25	
Location				
Peri-urban (*N* = 267)	212	79.4	1.00	0.07
Rural (*N* = 148)	106	71.62	0.65	
House_type				
Others (*N* = 15)	12	80	1.00	0.75
Cemented (*N* = 400)	306	76.5	0.81	
Religion				
Christianity (*N* = 281)	199	70.82	1.00	**0.0001**
Islam (*N* = 134)	119	88.81	3.27	
Occupation				
Skilled worker (*N* = 392)	300	76.53	1.00	0.85
Non-skilled worker (*N* = 23)	18	78.26	0.91	
Education				
Primary or none (*N* = 84)	73	86.9	1.00	**0.01**
Secondary or tertiary (*N* = 331)	245	74.02	0.43	
Total (*N* = 415)	318	76.63		

Table 2. Risk factor analysis of *Toxoplasma gondii* infection among women of childbearing age in the selected seven communities in Osun State, Nigeria.

Variables	Infection rate			
	N	%	OR	*p*-value
Contact with cats				
No (*N* = 385)	295	76.67	1.00	0.99
Yes (*N* = 30)	23	76.62	1.00	
Consuming raw meat				
No (*N* = 67)	57	85.07	1.00	0.07
Yes (*N* = 348)	261	75	0.53	
Consuming frozen meat				
No (*N* = 389)	297	76.35	1.00	0.67
Yes (*N* = 25)	20	80	0.81	
Unboiled water				
No (*N* = 38)	29	76.32	1.00	0.98
Yes (*N* = 375)	287	76.53	1.01	
Consuming raw vegetable				
No (*N* = 247)	188	76.11	1.00	0.76
Yes (*N* = 168)	130	77.38	1.07	
Handling raw meat				
No (*N* = 41)	31	75.61	1.00	0.87
Yes (*N* = 374)	287	76.74	1.06	
Hand washing				
No (*N* = 57)	44	77.19	1.00	0.9
Yes (*N* = 357)	273	76.47	0.96	
Miscarriage				
No (*N* = 337)	257	76.26	1.00	0.71
Yes (*N* = 78)	61	78.21	1.12	
Number of miscarriages				
No (*N* = 335)	255	76.12	1.00	0.74
1 (*N* = 41)	33	80.49	1.05	
>1 (*N* = 39)	30	76.92	1.01	
Total (*N* = 415)				

Table 3. Logistic regression analysis of *Toxoplasma gondii* infection among women of childbearing age in the selected seven communities in Osun State, Nigeria.

Variables	AOR	95% CI	*p*-value
Age (median = 27)			
≤27 (*N* = 209)	1.00		0.37
>27 (*N* = 206)	1.26	0.76–2.08	
Location			
Peri-urban (*N* = 267)	1.00		**<0.001**
Rural (*N* = 148)	0.39	0.23–0.69	
House_type			
Others (*N* = 15)	1.00		0.58
Cemented (*N* = 400)	1.51	0.36–6.43	
Religion			
Christianity (*N* = 281)	1.00		**<0.001**
Islam (*N* = 134)	4.71	2.43–9.15	
Occupation			
Skilled worker (*N* = 392)	1.00		0.55
Non-skilled worker (*N* = 23)	0.72	0.25–2.09	
Education			
Primary or none (*N* = 84)	1.00		0.06
Secondary or tertiary (*N* = 331)	0.48	0.23–1.02	
Contact with cats			
No (*N* = 385)	1.00		0.68
Yes (*N* = 30)	0.81	0.31–2.16	
Consuming raw meat			
No (*N* = 67)	1.00		0.18
Yes (*N* = 348)	0.56	0.25–1.29	
Consuming frozen meat			
No (*N* = 389)	1.00		0.87
Yes (*N* = 25)	1.11	0.34–3.56	
Unboiled water			
No (*N* = 38)	1.00		0.49
Yes (*N* = 375)	0.73	0.29–1.79	
Consuming raw vegetable			
No (*N* = 247)	1.00		0.63
Yes (*N* = 168)	1.13	0.69–1.87	
Handling raw meat			
No (*N* = 41)	1.00		0.94
Yes (*N* = 374)	0.97	0.41–2.28	
Hand washing			
No (*N* = 57)	1.00		0.57
Yes (*N* = 357)	0.80	0.37–1.73	
Miscarriage			
No (*N* = 337)	1.00		0.57
Yes (*N* = 78)	1.21	0.63–2.33	

Discussion

This study showed an overall seroprevalence of 76.63% among women of childbearing age in Osun State, Southwest Nigeria. The seroprevalence value in this study is significantly higher than prevalence of 44% reported in women of childbearing age from Benue State, Northcentral Nigeria [19], and also higher than those reported from healthy individual from different part of Nigeria which ranged from 20.83% to 41.13% [20–22]. However, it is lower than 85.54% reported among HIV-positive individual from Lagos State, Southwest Nigeria [23]. The high seroprevalence value recorded among the studied population could be as a result of inadequate personal hygiene of the women, nutritional habits or the favorable climatic conditions suitable for sporulation and survival of oocysts in the environment.

The seroprevalence of *T. gondii* obtained in this study is higher than 65.8% reported among women of childbearing age in Goiania-Go, Brazil [24], 47.8% among pregnant women in Algeria [25], 47.5% among childbearing women population in Isafahan Province, Iran [26], 43% among pregnant women in Morocco [27], 22.4% in women of childbearing age from India [28] and 41.20% in childbearing women from Western Romania [29]. The prevalence is also higher than 12.4% and 22.4% in women of childbearing age from two provinces Siena and Bari, in Italy [30], and also higher than 54.50% in women of the same population in Njinikom, Cameroon [31]. However, it is lower than 81.4% reported among women of childbearing age in Central Ethiopia [13]. A considerable number of studies have reported that several factors could be responsible for the wide variation in the seroprevalence of *T. gondii* infection in humans between countries and often within a specific country or between different communities in the same region including dietary habits, socioeconomic status, cultural habits, quality of water and sanitation coverage [32,33].

The *Toxoplasma* IgG-positive and IgM-negative results (44.10%; 183/415) suggest past exposure to the parasite, while positive results for IgM (6.02%, 25/415) are indicators of acute or recent exposures. Besides, childbearing age women who are negative for IgG and IgM antibodies (23.37%; 97/415) are at risk of primary infection and should be monitored for seroconversion in case they become pregnant [34]. Though positive IgM results are characteristic markers of recent infections, further confirmation to exclude reaction of natural IgM antibody with *Toxoplasma* antigen is needed [34]. IgM antibodies can persist for a long time with no risk of congenital infections.

In the current study, increase in seropositivity of *T. gondii* was observed as age increased, which agrees with previous studies among pregnant women [35–37]. This could be explained by the fact that older women are more likely to have been exposed to any one of the risk factors than younger women as a result of longer exposure time. This study also shows that women with a primary education or none had a significantly higher (86.9%) seroprevalence of *T. gondii* infection compared with women with a secondary or higher education (74.02%) ($p = 0.01$). The reason could have been knowledge about health education and maintenance of personal hygiene. Similar observation was reported in Taipei city, Taiwan among pregnant women where women with a senior high school educational level or lower had a higher seroprevalence of toxoplasmosis than those with a bachelor degree or higher [38].

In this study, living in rural area was associated with *T. gondii* infection (adjusted OR = 0.39; 95% CI: 0.23–0.69, $p = 0.001$). The positive association of the seroprevalence with the location (place of residence) may reflect the lifestyle of the people that makes them more predisposed to the infection. The activities carried out in rural areas may expose individuals to contaminated soil or water [39]. Farming, gardening and

handling animals are factors that may contribute to a higher prevalence in women residing in rural areas. In this study, we also found a positive association between childbearing women of Islam religion and *T. gondii* infection (adjusted OR = 4.71, CI; 2.43–9.15, $p = 0.001$). Since Islam religion forbids eating of certain meat (e.g. pork meat), which is one of the reservoirs for *T. gondii* tissue cyst, meat such as goat or chicken has also been implicated as sources of *T. gondii* infection. Hence, childbearing women of Islam faith could have contracted the infection through this route as the questionnaire survey revealed that 83.8% of the women responded to have consumed undercooked meat of chicken and cow. The other plausible explanation in these studied groups of women could also be attributed to drinking water, which might have been contaminated with cat's oocysts as greater proportion (90.4%) of the women claimed to have drunk unboiled water as revealed by the questionnaire. The rest of the socio-demographic and behavioral characteristics of the women and the environmental risk factors did not show any association with *T. gondii* infection. Our study has some limitations. First, we did not use avidity test that could have helped to determine if the *Toxoplasma* infection acquired by the women is of recent. It would have been ideal to include more communities, but this was not feasible due to financial constraints.

Conclusion

The present study provides new epidemiological data on the seroprevalence of *T. gondii* infection in women of childbearing age from selected communities in Osun State, Nigeria. Our results show a high seroprevalence of *T. gondii* infection (76.63%) in the studied population. Further studies should be conducted to determine additional risk factors that contribute to such high seroprevalence. This knowledge is important to predict and prevent the risk of infection in women of childbearing age and particularly of pregnant women as well in the future. The serological status of women of childbearing age provides valuable information on immunity, which may help to prevent congenital infection by identifying women at risk. The findings in this study may serve as a useful information for health policymakers for counseling and educational programs on toxoplasmosis during antenatal clinics, and for the implementation at the national level of a screening and prevention program for pregnant women.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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