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Seroprevalence of ocular toxoplasmosis in a major referral eye center in Southwest, Nigeria

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ABSTRACT

Objective: To assess the prevalence of ocular toxoplasmosis by seroprevalence of *Toxoplasma (T.) gondii* among people attending the referral eye center in Southwest Nigeria, and to identify associated risk factors and socio-economic determinants related to ocular toxoplasmosis.

Methods: A systematic questionnaire was used and blood samples of 3 mL each were taken. Serological tests of anti-*T. gondii* IgG and IgM antibodies were performed using the ELISA kit. Eye examinations were performed.

Results: *T. gondii* antibodies were detected in 52.6% of the tested patients. A total of 409 respondents were surveyed. The prevalence of toxoplasmosis increased with age which was statistically significant ($P=0.001$). The highest prevalence was found in farmers (69.2%, $P=0.011$). The overall prevalence of ocular toxoplasmosis within the study area was 1.7%.

Conclusions: The high seroprevalence recorded in this study is suggestive of the endemic nature of the disease. Awareness among the populace about *Toxoplasma* infection is imperative in understanding the epidemiology of the infection.

in the intestine and excreted through the feces of its specific host is the major route of infection[2]. The success of infection caused by *T. gondii* is based on a fragile equilibrium between the host immune response and the immune avoidance strategy elicited by the parasite which enables the ultimate survival of the parasite in the host[3]. Ocular toxoplasmosis may result from retinal infection with the protozoan, *T. gondii*. This parasite, which exists as multiple clonal subpopulations and in three stages, is capable of replication in any nucleated cell of its primary feline[4]. The most common agent causing retinal infection in the USA is *T. gondii*, with over one million individuals infected with the intracellular

Significance

Our findings have enhanced the current understanding about toxoplasmosis and ocular toxoplasmosis in the southwestern, Nigeria. This study has revealed that over half of the tested patients were positive to *Toxoplasma gondii* while the prevalence of ocular toxoplasmosis is relatively low. Notably, a significant portion of the population remains unaware of toxoplasmosis despite endemic presence in the studied region. Additionally, our study has also provided valuable insights for the management of patients with toxoplasmosis and ocular toxoplasmosis.

KEYWORDS: Toxoplasmosis; Antibodies; Seroprevalence

1. Introduction

Toxoplasmosis is a major cause of posterior uveitis in immunocompromised patients worldwide[1]. The causative organism is the protozoan parasite, *Toxoplasma (T.) gondii*, whose primary host is the cat. Oral ingestion of *T. gondii* oocysts produced

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parasite[5]. About 21 000 individuals of this number develop ocular toxoplasmosis, and nearly 5 000 manifest visual symptoms[5]. The frequency of ocular involvement is substantially greater in other parts of the world like southern Brazil where it occurs in 18% of individuals seropositive to *T. gondii* and the infection has been shown to be severe in the extremes of age and in the immune suppressed[6]. Factors such as parasite virulence host susceptibility and eating habits are considered to influence the variations seen in seroprevalence and visual outcomes between regions[7]. Although there are multiple studies in the literature reporting clinical aspects of the disease, most of them come from uveitis tertiary centers, where up to one-fourth of affected eyes may present with blindness and constitute more severe cases than community-based studies[6]. There is scarce information about the real-life frequency of ocular lesions, visual outcomes, and risk factors for poor prognosis of ocular toxoplasmosis. The prevalence of ocular involvement is markedly different between individuals with congenital and those with post-natal acquired infections. Even among those with post-natal acquired infections, age influences the risk and timing of ocular involvement. The severity of toxoplasma retinochoroiditis (in terms of lesion size, location, and associated inflammation) is also affected by patient age at the time of initial infection or recurrence[7]. Majority of previous studies on *T. gondii* infection in Nigeria and Africa had hitherto been predicated on women of reproductive age and HIV/AIDS patients[8,9]. There is paucity of information on ocular toxoplasmosis and etiology of posterior uveitis in Southwest, Nigeria. Therefore, this study probes into this field and determine the prevalence of *T. gondii* among patients in the eye center and its associated risks.

2. Materials and methods

2.1. Inclusion criteria

The following criteria were considered for inclusion of individuals in the present study: a) Subjects' confirmation of willingness to participate; b) Presence of clear media in both eyes to facilitate apparent ophthalmoscopic view of the fundi.

2.2. Sampling size

The minimum sample size required for the report of the prevalence of ocular toxoplasmosis subjects was given by the formula[10]:

$$N = Z^2(1-P)(P)/b^2$$

where N is minimum sample size, Z is standard normal deviate corresponding to α (level of significance) of 5% = 1.96, P = anticipated prevalence of ocular toxoplasmosis (estimated as 50%), b = desired error bound taken as 5%.

2.3. Materials for examination

The materials used for the study include Snellen's charts, illiterate E charts, occluders, pinholes, pen torch, 1% tropicamide eye drop, 2.5% phenylephrine eye drop, 2% pilocarpine eye drop, direct ophthalmoscope, indirect ophthalmoscope, +78DS lens, a Haag Streit slit lamp microscope mounted with recently calibrated Goldman applanation tonometer, plain sample bottles, cotton wool, methylated spirit, 23 gauge needle and syringe, centrifuge machine, Pasteur pipette, ELISA machine, ELISA kits, and TOPCON 2000 3D fundus camera.

2.4. Clinical examination

The clinical examination involves registering individuals who meet the inclusion criteria by collecting their personal and socio-demographic information. This process includes testing the presenting visual acuity of each eye using a Snellen chart, followed by further evaluation and examination of the eligible respondents. Visual acuity of each eye was tested with the respondent looking through a pinhole for those with visual acuity of less than 6/4. The ocular examination performed include anterior segment examination, tonometry and funduscopy.

2.4.1. Anterior segment examination

Anterior segment examination was achieved with the use of a pen torch and the eyelids, conjunctiva, cornea, anterior chamber, pupils, and lenses were examined.

2.4.2. Tonometry

Intraocular pressure was measured in each eye, by Goldmann Applanation tonometry (Haag-Streit) after application of topical anesthetic agent and fluorescein into the conjunctiva sac and cleaning of the tonometer head with sterile cotton swab. The end point was taken as when the inner margins of the semicircles were aligned. The average of two measurements were taken.

2.4.3. Fundoscopy

Indirect ophthalmoscopy was performed on the participants through dilated pupils. Pupillary dilation was achieved with 1% tropicamide eye drop and 2.5% phenylephrine eye drop. Fundus picture was taken in those with toxoplasma scar.

2.5. Blood sample collection and handling

Venous blood of 3 mL was collected from each participant by a phlebotomist using a 23-gauge sterile needle and syringe and dispensed into a labeled plain test tube after cleaning the site with cotton wool swab soaked in spirit. The samples were centrifuged at 1000 rpm for 3 min to separate serum. Clear serum was collected

with Pasteur pipette into labeled plain test tube and stored at -20°C in the virology laboratory for further analysis. Each serum sample were tested with ELISA test kits (DIALAB(R) GmbH, Co. Austria) following manufacturer's protocols. The sampling period was a period of four months.

2.6. *Toxoplasma serology*

Frozen sera were allowed to thaw at room temperature. Thawed sera were then tested in duplicates for *anti-T. gondii* IgG antibody with the use of commercially procured Enzyme Linked Immunosorbent Assay (ELISA) Kits (DIALAB(R) GmbH, Co. Austria) for specific *anti-T. gondii* IgG and IgM antibodies according to the manufacturer's instruction.

2.7. *Questionnaire administration*

A total of 409 consecutive patients were administered an interviewer-led questionnaire to gather personal information regarding their biodata and risk factors for toxoplasmosis infection, as well as the onset of visual and systemic symptoms. This was followed by a clinical ocular examination.

2.8. *Data analysis*

Data collected were entered into a data base and analyzed using SPSS version 21 Statistical software. The data were then cleaned and frequencies and means were generated to observe varying patterns of distribution among the respondents. Fisher's exact test was used to determine associations between ocular toxoplasmosis and risk factors while the *Chi-square* test was used to determine significant differences in seropositivity. The *t*-test was used to compare mean ages of individuals with ocular lesions and those without. Significance level was set at 5%.

2.9. *Consent and ethical approval*

Individual written informed consent was obtained from all the participants and the ethical approval (No.NHREC/18/08/2016) and clearance for the study was also obtained from the ethical committee.

3. Results

3.1. *Socio-demographic profile of study participants*

A total number of 409 patients were examined for toxoplasmosis during the study period, among which 170 of the respondents (41.6%) were male and 239 (58.4%) were female. The age of all

respondents ranged from 14 to 97 years, with a mean age of 57.2 ± 17.9 years. Majority of the respondents were Christians (90.7%) and 86.8% of them from Yoruba ethnic extraction. Two thirds (65.8%) of the respondents were married. About one third (31.5%) of the respondents were traders while a quarter (22.5%) were civil servants. Approximately one-third of the respondents had completed tertiary education (35.2%), while 28.1% had attained secondary education, as shown in Table 1.

Table 1. Socio-demographic profile of study participants.

Variables	Frequency (<i>n</i> = 409)
Sex	
Male	170 (41.6)
Female	239 (58.4)
Age, years	
Mean \pm SD	57.2 ± 17.9
<20	16 (3.9)
20 – 39	108 (26.4)
40 – 59	141 (34.5)
60 – 79	122 (29.8)
80 – 99	22 (5.4)
Religion	
Christianity	371 (90.7)
Islam	38 (9.3)
Ethnic group	
Yoruba	355 (86.8)
Igbo	18 (4.4)
Hausa	2 (0.5)
Others	34 (8.3)
Level of education	
None	86 (21.0)
Primary	64 (15.6)
Secondary	115 (28.1)
Tertiary	144 (35.2)
Marital status	
Married	269 (65.8)
Divorced	10 (2.4)
Separated	57 (13.9)
Widow(er)	13 (3.2)
Single	60 (14.7)
Occupations	
Civil servant	92 (22.5)
Trader/business man	129 (31.5)
Artisan	38 (9.3)
Farmer	39 (9.5)
Student	40 (9.8)
Unemployed	23 (5.6)
Retired	42 (10.3)
Clergy	6 (1.5)

Data are expressed by *n* (%).

3.2. *Seroprevalence of toxoplasmosis infection*

The serological test revealed that about half (52.6%) of the respondents had toxoplasmosis infection. The study shows that seven (1.7%) out of the total respondents were positive for ocular lesion.

3.3. Association between some socio–demographic factors and toxoplasmosis infection

As suggested by Table 2, the prevalence of toxoplasmosis is higher among male respondents than females but the difference in the prevalence was not statistically significant ($P=0.257$). The prevalence of toxoplasmosis differs according to age, as the infection with toxoplasmosis was found to increase with age. The highest prevalence was recorded among the age group 80–99 years (68.2%), while the lowest (37.5%) was among respondents less than 20 years of age; and this difference was found to be statistically significant ($P<0.001$). The highest prevalence was recorded among farmers (69.2%) followed by retired civil servants (69.0%), while the lowest was among the students (40.0%) and this difference was found to be statistically significant ($P=0.019$).

Table 2. Association between some socio-demographic factors and toxoplasmosis infection.

Variables	Respondents tested (n = 409)	Positive to IgG (n = 215)	P
Sex			
Male	170	95 (55.9)	0.257
Female	239	120 (50.2)	
Age, years			
<20	16	6 (37.5)	<0.001
20 – 39	108	41(38.0)	
40 – 59	141	6 (53.9)	
60 – 79	122	77 (63.1)	
80 – 99	22	15 (68.2)	
Occupations			
Civil servant	92	48 (52.2)	0.019
Trader/Businessman	129	57 (44.2)	
Artisan	38	23 (60.5)	
Farmer	39	27 (69.2)	
Students	40	16 (40.0)	
Unemployed	23	11 (47.8)	
Retired civil servants	42	29 (69.0)	
Clergy	6	4 (66.7)	

Data are expressed by n (%).

3.4. Distribution of toxoplasmosis infection according to type of meat consumed

The prevalence of toxoplasmosis infection was found to be higher among consumers of various types of meat (beef, pork, suya, chevon, chicken, and fish) compared to non-consumers. However, the differences in prevalence across all meat types were not statistically significant (Table 3).

Table 3. Distribution of toxoplasmosis infection according to type of meat consumed by the respondents.

Variables	Respondents tested (n = 409)	Positive to IgG (n = 215)	Statistical tests
Consumption of beef			
Yes	401	213 (53.1)	$\chi^2 = 2.574$ $P = 0.109$
No	8	2 (25.0)	
Consumption of pork			
Yes	72	44 (61.1)	$\chi^2 = 2.558$ $P = 0.110$
No	337	171 (50.7)	
Consumption of suya			
Yes	74	46 (62.2)	$\chi^2 = 3.336$ $P = 0.068$
No	335	169 (50.4)	
Consumption of chevon			
Yes	82	51 (62.2)	$\chi^2 = 3.336$ $P = 0.068$
No	327	164 (50.2)	
Consumption of chicken			
Yes	83	50 (60.2)	$\chi^2 = 2.459$ $P = 0.117$
No	326	165 (50.0)	
Consumption of fish			
Yes	321	175 (54.5)	$\chi^2 = 2.275$ $P = 0.131$
No	88	40 (45.5)	

Data are expressed by n (%); * Likelihood ratio.

3.5. Prevalence of toxoplasmosis according to sources of water for domestic purposes

There was no statistically significant difference in the prevalence of toxoplasmosis among the users and non-users of the different sources of water (Table 4).

Table 4. Prevalence of toxoplasmosis according to sources of water for domestic purposes.

Variables	Respondents tested (n = 409)	Positive to IgG (n = 215)	Statistical tests
Consumption of bottled water			
Yes	23	13 (56.5)	$\chi^2 = 2.459$ $P = 0.117$
No	386	202 (52.3)	
Consumption of well water			
Yes	223	120 (53.8)	$\chi^2 = 0.305$ $P = 0.581$
No	186	95 (51.1)	
Consumption of sachet water			
Yes	144	81 (56.3)	$\chi^2 = 1.209$ $P = 0.272$
No	265	134 (50.6)	
Consumption of pipe borne water			
Yes	43	27 (62.8)	$\chi^2 = 2.014$ $P = 0.156$
No	366	188 (51.4)	
Consumption of rainwater			
Yes	101	28 (59.6)	$\chi^2 = 1.046$ $P = 0.306$
No	308	187 (51.7)	
Consumption of borehole water			
Yes	47	50 (49.5)	$\chi^2 = 0.504$ $P = 0.478$
No	362	165 (53.6)	

Data are expressed by n (%).

3.6. Prevalence of toxoplasmosis infection according to methods of handling meat products for consumption, involvement in backyard gardening and presence of rodents/cockroaches in homes

As suggested by Table 5, there is no statistically significant association between methods of handling meat products for consumption and seroprevalence of toxoplasmosis infection. Although there was a higher prevalence of toxoplasmosis infection as the frequency of washing of hands after handling raw meat reduced, while difference was not statistically significant ($P=0.384$). Also, the prevalence was higher among respondents who did not wash their hands before meal (77.8%) than who did (51.9%), but there was no statistically significant difference ($P=0.173$). The respondents who engaged in backyard gardening had higher prevalence of the disease but the difference was not statistically significant ($P=0.430$). Likewise, patients reporting the presence of rodents and cockroaches in their homes had a higher prevalence of toxoplasmosis (53%) but this was not statistically significant ($P=0.200$).

Table 5. Prevalence of toxoplasmosis infection according to methods of handling meat products, involvement in backyard gardening and presence of rodents/cockroaches in homes.

Variables	Respondents tested (n = 409)	Positive to IgG (n = 215)	Statistical tests
Tasting of meat while cooking			
Yes	129	63 (48.8)	$\chi^2 = 1.051$
No	280	152 (54.3)	$P = 0.305$
Washing knife after cutting raw meat			
Always	358	182 (50.8)	$\chi^2 = 3.507$
Sometimes	40	26 (65.0)	$P = 0.173$
Never	11	7 (63.6)	
Washing hands after handling raw meat			
Always	343	176 (51.3)	$\chi^2 = 1.913$
Sometimes	48	27 (56.3)	$P = 0.384$
Never	18	12 (66.7)	
Washing hands before meal			
Yes	399	207 (51.9)	$\chi^2 = 3.507$
No	10	9 (77.8)	$P = 0.173$
Backyard gardening			
Yes	215	117 (54.4)	$\chi^2 = 0.623$
No	194	98 (50.5)	$P = 0.430$
Presence of rodents/cockroaches			
Yes	402	213 (53.0)	$\chi^2 = 1.645$
No	7	2 (28.6)	$P = 0.200$

Data are expressed by n (%).

3.7. Ownership of domestic animals and prevalence of toxoplasmosis infection

Among the owners of domestic animals, ownership of turkey, dog, pig was associated with increased prevalence of toxoplasmosis infection, while it was only ownership of turkey that the difference in the prevalence among owners and non-owners was statistically significant ($P=0.029$), as suggested by Table 6.

Table 6. Relationship between ownership of domestic animals and prevalence of toxoplasmosis infection.

Variables	Respondents tested (n = 114)	Positive to IgG (n = 71)	Statistical tests
Ownership of chicken			
Yes	90	43 (47.8)	$\chi^2 = 0.224$
No	54	28 (51.9)	$P = 0.636$
Ownership of turkey			
Yes	16	12 (75.0)	$\chi^2 = 4.754$
No	128	59 (46.1)	$P = 0.029$
Ownership of dogs			
Yes	38	20 (52.6)	$\chi^2 = 0.228$
No	106	51 (48.1)	$P = 0.633$
Ownership of pigs			
Yes	4	2 (50.0)	$\chi^2 = 0.001$
No	140	69 (49.3)	$P = 0.978$
Ownership of goats			
Yes	35	13 (37.1)	$\chi^2 = 2.737$
No	109	58 (53.2)	$P = 0.098$
Ownership of cats			
Yes	7	3 (42.9)	$\chi^2 = 0.123$
No	137	68 (49.6)	$P = 0.726$

Data are expressed by n (%).

4. Discussion

Toxoplasmosis, zoonotic disease of global distribution had attracted significant attention by scientist in the last few years. Most pathological conditions associated with toxoplasmosis are primarily caused by tachyzoites that undergo active differentiation in the tissues[11]. Toxoplasma specific antibodies (IgG) were detected in 52.6% of the eye patients examined and clearly indicating that approximately more than half of those tested had previous experience with *T. gondii* infection. This result is similar to the findings of Prestes-Carneiro *et al.* who reported *T. gondii* in 52.6% of Dona Carmen inhabitants in Sao Paulo, Brazil[12]. Previous report indicated that the prevalence of *T. gondii* infection vary according to the local community, continents, and biological characters[13]. The high seroprevalence recorded in this study is suggestive of the endemic nature of the disease, variations in environmental and geographical factors in Southwest, Nigeria. Similar findings by

Tete-Benissan *et al.*, reported a prevalence of 59.7% in Togolese community while Ekanem *et al.*, revealed seroprevalence rate of 55.8% in Southern Nigeria[14,15]. These reports conformed to the findings of this study.

The males examined in the present study recorded a significantly higher seropositivity to anti-*T. gondii* IgG antibodies compared to the female patients. The male preponderance could be attributed to the fact that males are more exposed to the animals and soil. To the best of our knowledge, findings from this study was probably the first report on the seroprevalence of *T. gondii* antibodies among the eye clinic patients in the Southwest, Nigeria, as a previous study by Olurin screened eye patients using the dye test[16]. The trend of seropositivity of anti-*T. gondii* antibodies among the respondents was age dependent. This agrees with Olusi *et al.*[17]. The reason for this may not be unconnected with constant exposure to risk factors of toxoplasmosis and high level of illiteracy among the population. The highest seropositivity to Toxoplasma infection recorded among the farmers (69.2%) could be due to their constant exposure to soil that might have been contaminated by cat's feces[13]. These variations in seroprevalence of *T. gondii* may probably be related to the lifestyle of these cats, the breeds, environmental and geographical factors as well as the type of serological tests used in detection of *T. gondii* antibodies[3]. This was also in conformity with the findings of Deji-Agboola *et al.* that farmers are at greater risk of being infected with *T. gondii* than others belonging to other occupational groups[18].

Except for Turkey there was no association between the ownership of domestic animals and *T. gondii* infection. This was surprising since meat from many animals other than turkey and ownership of domestic animals other than turkey have been documented more often as a cause of *T. gondii* infection. This finding is suggestive of further investigation to clear this paradox. Even more

surprising is the suggestion by Alvarado-Esquivel *et al.*[19] that turkey meat consumption might contribute to the acquisition of *T. gondii* infection. Although other authors have documented an association between *T. gondii* infections and consumption of raw meat[20], presence of rodents and cockroaches, sources of water, and backyard gardening[19], however, this study did not observe any association between these risk factors and seroprevalence of *T. gondii* IgG among the eye patients.

Globally, the seroprevalence of *T.gondii* varies greatly between (10%-90%). There is also variation between different countries and even within countries[21]. However, in most of these studies, molecular techniques have been used for defining cases[7]. Toxoplasmosis serology is useful to confirm active toxoplasmosis retinochoroiditis and to support clinical diagnosis. In over 50% of the immunocompetent patients, clinical signs of toxoplasmosis are apparent; therefore, assessing the serological status is essential in cases at high risk for more severe disease forms[7]. One of the patients with ocular toxoplasmosis in this study had a Toxoplasma scar located on the macula in the right eye, which is the region of keenest vision, leading to loss of vision which is a devastating complication of ocular toxoplasmosis. Though the prevalence of ocular toxoplasmosis appears low in the present study, the visual outcome of the affected individuals was poor due to *T. gondii* scars.

The prevalence of ocular toxoplasmosis in this study is 1.7%. This is consistent with reports from previous studies including Jones *et al.*[22] who recorded seroprevalence of ocular toxoplasmosis to be 2.0% in the United States, and Abu *et al.*[10] who discovered a prevalence rate of 2.6% in Ghana while Abraham *et al.*[23] documented a seroprevalence of 0.28% in Southern Nigeria. This clearly depicted seroprevalence of ocular toxoplasmosis in various parts of the world. The level of awareness and sensitization of the parasite, symptoms, risk factors and how infection affects

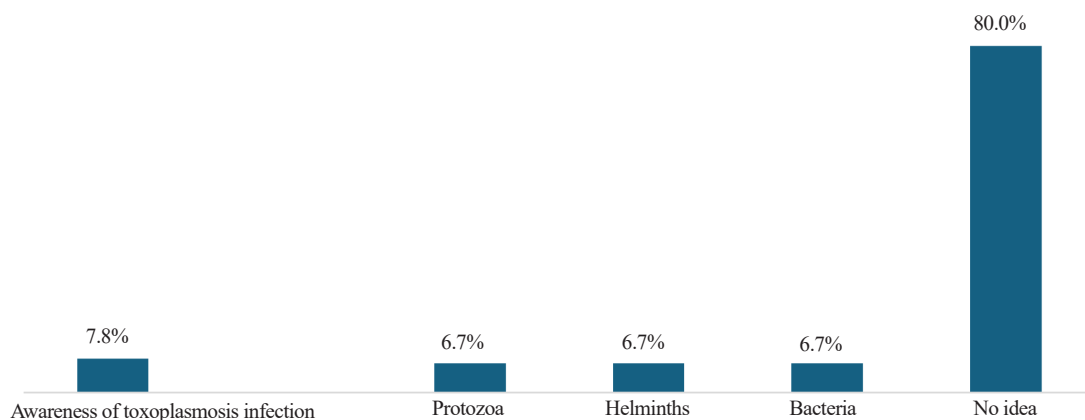


Figure 1. Awareness of toxoplasmosis infection and knowledge of the causes of toxoplasmosis infection.

the eyes were low. Findings revealed that about 92.2% had no knowledge of toxoplasmosis while about 80.0% did not know the etiology of the disease (Figure 1) and none had ever been screened for toxoplasmosis despite its endemic nature in our environment according to the results from questionnaire. The reason for this may not be unconnected with knowledge base of medical practitioners in this part of the world about the epidemiology of the disease and that it is dangerous to their community[8]. However, studies were carried out by medical practitioners in identifying this knowledge gap[8]. This limited awareness is consistent with previous reports from the USA[9]. These limitations in sensitization and awareness are also in line with previous reports of Fuh *et al*[1] and Ebrahimi *et al*[24]. About 6.7% of the total volunteered eye patients thought that the etiological agent of toxoplasmosis is a bacterium, helminth or protozoan while 80.0% claimed ignorance of the cause of the disease.

5. Conclusions

This study concluded that among the study population, although there is a high seroprevalence level of *T.gondii* infection, the prevalence of ocular toxoplasmosis is relatively low. Also, the knowledge and awareness of *T.gondii* infection among the respondents was very poor and none of the respondents had ever been screened for toxoplasmosis infection despite its endemic nature. These findings highlight the need for increased public awareness and screening programs to address the potential health risks associated with this infection.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Adegbehingbe SA, Olusi TA, Oniya MO and Fagbemi AT completed the literature review. Adegbehingbe SA collected the epidemiological and clinical data from the hospital. Fagbemi AT analyzed the data. Adegbehingbe SA, Olusi TA, Oniya MO and Fagbemi AT wrote the manuscript equally. Adegbehingbe SA provided funding and coordinated all sectors to provide great supports to this research.

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