

Seroprevalence and Risk Factors of Toxoplasmosis among Pregnant Women Attending Antenatal Clinic in Kaduna Metropolis and Environs

**Halima Sadia Bello^{1*}, Yahaya Abdullahi Umar¹, M. S. Abdulsalami¹
and Victor Oluwasanmi Amusan¹**

¹*Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors HSB, YAU and MSA designed the study, wrote the protocol and first draft of the manuscript. Authors HSB and VOA collected the specimens and managed the literature searches. All authors managed the analyses of the study, read and approved the final manuscript.

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ABSTRACT

Toxoplasmosis due to infection by *Toxoplasma gondii* is estimated to affect one third of the global population. In pregnancy, *T. gondii* infection represents the risk to induce miscarriage and congenital transmission. This study was therefore conducted to determine the seroprevalence of anti-*T. gondii* IgM antibodies among pregnant women attending antenatal clinics in Kaduna south, Nigeria. Enzyme-linked immunosorbent assay (ELISA) was used and structured questionnaire to obtain information on risk factors associated with infection among pregnant women attending antenatal clinics at Gwamna Awam General Hospital Kakuri and Yusuf Dantsoho Memorial Hospital Tudun Wada in Kaduna State. A total of One hundred and ninety two (192) blood samples (96 from each of the selected hospital) were collected from the pregnant women and screened using specific anti-*Toxoplasma gondii* IgM antibodies kit (IBL- International GMBH a Tecan Group Company). Result obtained from the 192 women serum analysed showed an overall seroprevalence of 31.3%

*Corresponding author: Email: Hbello064@gmail.com;
Email: voaplus@yahoo.com

using *T. gondii* specific IgM antibodies. Seropositivity was found to be statistically associated with age ($P < 0.05$) while Relative Risk (RR) analysis showed that women aged between 31-35 yrs. were found to have higher risk of contracting toxoplasmosis than all other age groups (RR 4.620). Chi-Square test showed that pregnancy stages, previous history of miscarriage and educational level of pregnant women were found to significantly influence prevalence of *T. gondii* infection ($P < 0.05$). Women with previous history of miscarriage was found to be more likely to be infected with toxoplasmosis than women with no history of miscarriage (RR= 1.278). Risk factor such as keeping cats was found to significantly influence Toxoplasma infection among the sampled women. The results of this study justifies the need to include laboratory testing for *T. gondii* infection during antenatal investigations and to educate women about the parasite in order to prevent maternal and subsequent congenital infections that might have been provoked *in-utero*.

Keywords: *Toxoplasma gondii*; seroprevalence; antibodies; risk factors; pregnant women.

1. INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that causes toxoplasmosis in human and other warm blooded animals. The coccidium parasitizes member of the cat family as its definitive host and has a wild range of intermediate hosts including humans [1]. Globally, about one third of the world's human population is estimated to carry *Toxoplasma gondii* parasite [2]. Toxoplasmosis is one of the most prevalent and widespread parasitic infections, it is often neglected especially in developing countries. Although it has long been reported to be widespread in many countries in West Africa [3]. The infection is usually mild and asymptomatic causing cold and flu in immunocompetent individuals but can rise to cervical lymphadenopathy, mononucleosis-like syndrome or inflammation of the choroid in up to 20% of infected persons; and in immune deficient individuals, such as acquired immune deficiency syndrome (AIDS) patients. Primary or reactivated infection can also result in encephalitis, pneumonia and inflammation of the heart muscles [4]. Toxoplasmosis has been reported as the commonest opportunistic infection in HIV/AIDS patient in developed and developing countries [5]. Worldwide, toxoplasmosis is reported to occur in all countries with seropositivity rates ranging from less than 10% to over 90% [6].

Association of toxoplasmosis to congenital impairment has recently been affirmed [7]. Consequences of primary congenital infection in pregnant women with normal immunity include miscarriages, still birth, blindness, deafness, mental retardation, microcephalus, hydrocephalus and other neurological diseases in the fetus [8]. Toxoplasmosis also enhances

mother-child transmission of Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV) [9].

Approximately, half a billion humans have antibodies to *Toxoplasma gondii* [10]. It has been reported that about 30-40% adult humans in the United States have antibodies to *T. gondii* [11,12,13]. Apparently, a healthy immune system may keep the parasite in check [14]. Considerable variations in seroprevalence of toxoplasmosis have been reported in diverse regions and in different individuals which are mainly related to factors that expose the population to the infected cysts. Age, occupation, sanitary conditions, educational status, contact with cat, soil, ruminant animals and location have been the main determinant risk factors of infection by *T. gondii* [15].

Infection due to *Toxoplasma gondii* is a worldwide zoonosis. The organism infects herbivorous, omnivorous and carnivorous animals, including birds [16,17]. Two main routes of transmission have been described in humans viz: oral-ingestion of the parasite (cyst) and through placental transmission to the fetus in pregnant women. The oral routes involve the ingestion of raw or undercooked meat that contains cysts or the ingestion of water or food contaminated with oocysts [18,19,20]. Infection of some women with the habit of tasting raw meat such as paste from chopped pork for meat balls during cooking has been reported [21]. Transmission through contaminated soil is also possible in humans by accidental ingestion of soil contaminated with *T. gondii* oocysts.

In human infection, *T. gondii* elicit immune response which can be detected by several serological tests including enzyme linked immunosorbent assay (ELISA). Immunoglobulin

G (IgG) antibodies usually appear within 1–2 weeks of acquisition of the infection, peak within 1–2 months, decline at various rates, and usually persist for life. It has been observed that the functional affinity of specific IgG antibodies is initially low after primary antigenic challenge and that it increases during subsequent weeks and months by antigen-driven B cell selection. Protein-denaturing reagents including urea are used to dissociate the antibody-antigen complex.

Immunoglobulin M (IgM) antibodies may appear earlier and decline more rapidly than IgG antibodies. An IgM test is still used by most laboratories to determine if a patient has been infected recently or in the distant past. In people with recently acquired primary infection, *T. gondii*-specific IgM antibodies are detected initially, and in most cases, these titers become negative within a few months. However, in some people, positive *T. gondii*-specific IgM titers can still be observed during the chronic phase of infection [22]. Some investigators have reported that IgM antibodies can be detected as long as 12 years after the acute infection [23]. The persistence of these IgM antibodies does not appear to have any clinical relevance, and these patients should be considered chronically infected.

Immunoglobulin A (IgA) antibodies may be detected in sera of acutely infected adults and congenitally infected infants and may persist for many months or more than a year [12]. Immunoglobulin E (IgE) antibodies are detectable in sera of acutely infected adults, congenitally infected infants, and children with congenital toxoplasmic chorioretinitis [24,25]. The duration of IgE seropositivity is shorter than IgM or IgA antibodies and hence appears useful for identifying recently acquired infections [26,25]. In Kaduna state like most parts of Nigeria, toxoplasmosis is not part of the routine screening test performed on pregnant women during antenatal clinic program in public hospitals. This study aimed to provide information on the status of toxoplasmosis and its associated risk factor among pregnant women in Kaduna metropolis and environs to guide policy makers in taking a proactive decision.

2. MATERIALS AND METHODS

2.1 Study Area

The research was conducted in Kaduna metropolis, Kaduna metropolis is the capital of

Kaduna State. It is located between latitudes $10^{\circ} 22' 00''$ - $10^{\circ} 40' 00''$ N and longitudes $70^{\circ} 20' 00''$ - $70^{\circ} 28' 00''$ E [27]. The metropolis occupies an area of about 260 km², and the distance between the eastern and western limits of the city is approximately 13.7 km.

2.2 Study Design

The research is a descriptive cross sectional study. Women attending antenatal clinics of Gwamna Awam General Hospital Kakuri and Yusuf Dantsoho Memorial Hospital Tudun Wada were recruited for the study. All women were interviewed using a structured questionnaire with relevant information recorded for each woman. The questionnaires were administered to obtain information on important variables such as age, stage of pregnancy, occupation, and educational status of the women.

2.3 Selection of Hospitals

Gwamna Awam General Hospital, Kakuri and Yusuf Dantsoho Memorial Hospital, Tudun Wada Kaduna located in Kaduna South LGA of Kaduna State were selected by simple random techniques. The 2 hospitals provide medical and antenatal services to inhabitants of Kaduna metropolis and environs.

2.4 Target Population

The targeted population for the study were pregnant women of all ages attending antenatal clinic at Gwamna Awam General Hospital, Kakuri and Yusuf Dantsoho Memorial Hospital, Tudun Wada within Kaduna metropolis and environs.

2.5 Sample Size

One hundred and ninety two (192) samples (96 from each of the selected hospital) from pregnant women attending antenatal who have voluntarily given their consent to participate in the study was used.

2.6 Ethical Permission

Approval to conduct the research was obtained from the Kaduna State Ministry of Health Ethical Committee before sample collection commenced in the 2 State hospitals. The aim of the study was also clearly explained to pregnant women attending antenatal clinic in the selected health facilities in order to get their informed consent.

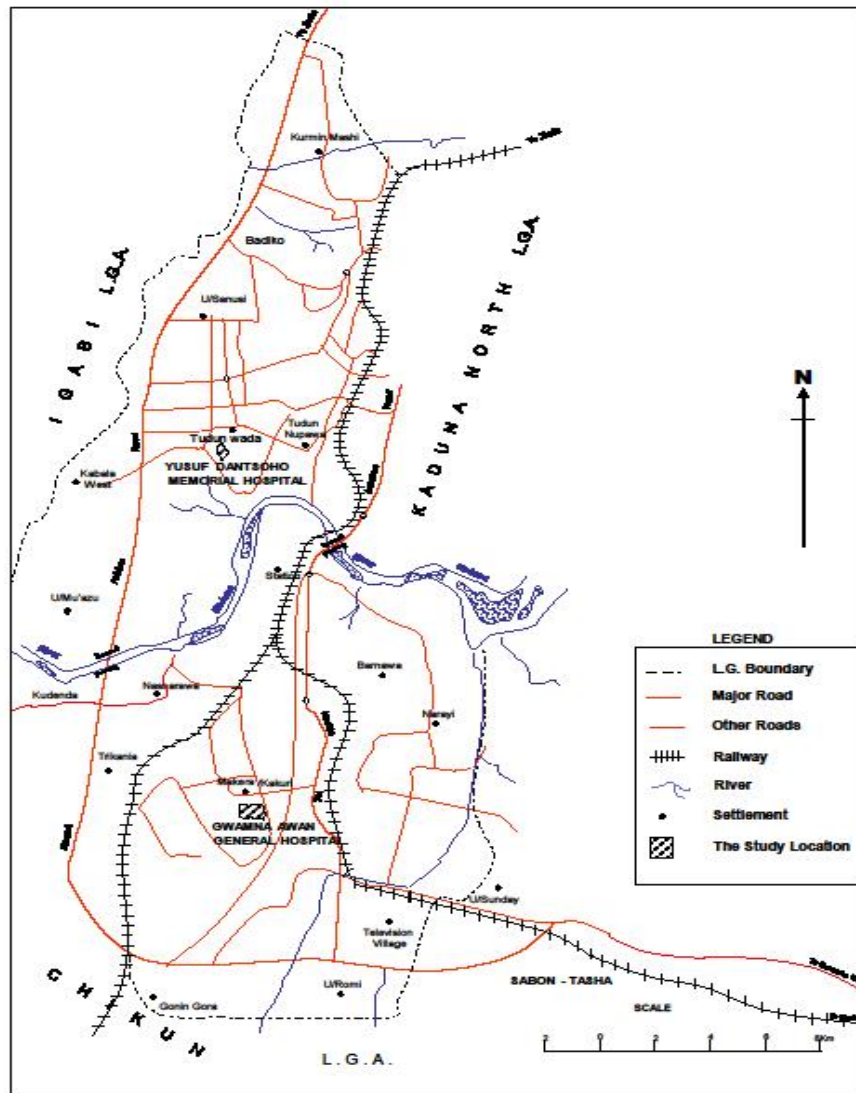


FIG. 1: KADUNA SOUTH LOCAL GOVERNMENT AREA SHOWING THE STUDY LOCATION.
 Source : Geo. Dept. NDA, Kaduna (2017)

Fig. 1. Map of Kaduna South LGA showing the study location

Women who do not consent were excluded from the study. To ensure confidentiality of subjects, names of respondents were not recorded during sample collection. Code of ethics on the use of human subjects in research as outlined in the NDA Research and Development Policy, 2016 was also adhere to strictly.

2.7 Questionnaire Administration

Information on the patients' age, previous abortion, and stage of pregnancy were collected

at the point of sample collection. The questionnaire was interpreted in local language for those who could not understand English.

2.8 Blood Sample Collection

Blood samples were collected from participants at the public health facilities with the help of medical personnel of the hospitals by venepuncture. Samples collected from each subject was transferred into a sterile labelled specimen bottle. Samples collected were then

centrifuged at 1000 rpm for 3 minutes to separate serum from blood cells. Serum sample obtained and stored in a thermox box were separately transferred to labelled fresh sample bottles, and later transported to Veterinary Teaching Hospital Laboratory (VTH/LAB), Faculty of Veterinary Medicine, Ahmadu Bello University Zaria for analysis. Serum samples were kept at – 20°C until analysed (All procedures were implemented to prevent sample contamination with animal materials).

2.9 Enzyme-Linked Immunosorbent Assay

Commercial Kits for specific anti-*Toxoplasma gondii* IgM antibodies were purchased and was used according to the Manufactures instruction (IBL- International GMBH a Tecan Group Company).

2.10 Detection of *Toxoplasma gondii* Antibody

Antibody ELISA technique was employed, strictly as instructed by manufacturer. One in forty (1:40) dilutions of specimen, negative control, positive control and calibrator were prepared by adding 5 µl of the aforementioned separately to 200 µl of sample diluent and mixed well. One hundred microliter (100 µl) of diluted sera, calibrator and controls were dispensed into appropriate wells. One hundred microliter (100 µl) of absorbent solution was dispensed in 1A well position for reagent blank. The holder was tapped to remove air bubbles from the liquid and was mixed well and incubated for 30 min at room temperature. Liquid from each of the wells was removed and washed three times repeatedly with washing buffer. One hundred microliter (100 µl) of enzyme conjugate was dispensed into each well and incubated for 30 min at room

temperature. The enzyme conjugate was then removed from all the wells and washed repeated three times with washing buffer. One hundred microliter (100 µl) of TMB Chromogenic Substrate was then dispensed to each well and again incubated for 15 minutes at room temperature. To stop the reaction, 100 µl of 2NHCl was added and results was read using an automated ELISA machine.

2.11 Statistical Analysis

Descriptive statistical analysis was performed using SPSS 17.0. All statistical analyses were carried out using a 95% confidence interval, a significance level of $P < 0.05$ and Chi-Square test of independence was carried out for all contingency table. Relative risk (RR) was evaluated for significant risk factors. Questionnaires administered were evaluated to determine the correlation between seroprevalence and several risk factors using Two-tailed test of significance.

3. RESULTS

The overall prevalence of anti-toxoplasma antibodies detected by ELISA is presented in Table 1. Out of 192 pregnant women sampled from Gwamna Awam General Hospital Kakuri and Yusuf Dantsoho Memorial Hospital T/Wada, 31.3% showed evidence of toxoplasma infection. The highest prevalence of 39.6% was recorded among pregnant women at Yusuf Dantsoho Memorial Hospital T/Wada while relatively lower prevalence (22.9%) was recorded among women that attended antenatal clinic at Gwamna Awam General Hospital Kakuri. Statistical analysis indicated significant differences in seroprevalence of toxoplasmosis among pregnant women in the 2 health facilities ($P < 0.05$).

Table 1. Overall prevalence of toxoplasmosis

Health Facility	Number of subject examined	Number of subject positive	Percentage (%) of subjects positive
GwamnaAwam General Hospital Kakuri	96	22	22.9
Yusuf Dantsoho Memorial Hospital Tudun Wada	96	38	39.6
Total	192	60	31.3

$$\chi^2 = 14.267, Df = 1, P\text{-value} = 0.039$$

Table 2 showed the seroprevalence of toxoplasma infection based on age of subjects sampled. The highest evidence of toxoplasma infection was recorded in 31-35 yr. age group with a seroprevalence of 67.7%. This was followed by women within 21-25, > 40 and 36-40 yr. age groups with seroprevalences of 34.6%, 28.5% and 27.8% respectively. The least prevalence (17.9%) of toxoplasma infection was recorded in pregnant women aged below 20 yrs. Among the different age groups, women aged between 31-35 yr. age group were found to have higher risk of contracting toxoplasmosis than all other age groups (RR 4.620).

Out of the 192 pregnant women tested, 64 were in their 1st trimester, 96 in 2nd trimester and 32 in 3rd trimester (Table 3). Pregnant women in their 2nd trimester showed the highest evidence of toxoplasma infection with seroprevalence of 35.4%, followed by women in their 3rd and 1st trimesters with seroprevalence of 31.3% and 25.0%. Statistical analysis showed a significant difference between trimesters with respect to anti-toxoplasma antibodies ($P < 0.05$).

From a total of 192 pregnant women examined, 149 had no history of miscarriage, 33 had 01 case of miscarriage while 10 have had more than 1 case of miscarriage (Table 4). The highest seroprevalence of toxoplasma infection was recorded in women who had one (1) history of miscarriage once (48.5%), followed by women that had more than 1 miscarriages in their life time with seropositivity of 40%. However,

pregnant women with no history of miscarriage had significantly lower seroprevalence of 26.8%. Chi-Square analysis indicated a strong association between previous history of abortion and the presence of antibodies to toxoplasma infection ($P < 0.05$). Women with previous history of miscarriages are about 2x more likely to be infected with toxoplasmosis than women with no history of miscarriage (RR= 1.278)

Among the 192 pregnant women screen for toxoplasma antibodies, 111 had primary school certificate, 36 had secondary school certificate, 24 had tertiary certificate while 24 had no formal education (Table 5). The highest seropositivity of 37.8% was recorded among pregnant women who had only primary education, this was followed by women who had tertiary and secondary education with seroprevalence of 28.6% and 22.2% respectively. However, relatively lower seropositivity (16.7%) was recorded among pregnant women that had no formal education. Chi-Square test indicated a strong association between contracting toxoplasma infection and educational level of pregnant women in the study area ($P < 0.05$).

The seropositivity of toxoplasmosis was found to be significantly higher (35.1%) among pregnant women that do not keep cats than women who keep cats (22.9%) (Table 6). Analysis of the data revealed that there was a significant statistical difference between women who keep cats and those who do not with regards to toxoplasma infection ($P < 0.05$).

Table 2. Seroprevalence of *T. gondii* in relation to age of sampled women

Age	Number of subject examined	Number of subject positive	Percentage (%) of subjects positive	Relative risk (RR)
<20	39	7	17.9	0.481
21-25	26	9	34.6	1.165
26-30	71	16	22.5	0.640
31-35	31	21	67.7	4.620
36-40	18	5	27.8	0.846
>40	7	2	28.5	0.880
Total	192	60	31.3	

$$X^2 = 25.600, Df = 5, P\text{-value} = 0.00$$

Table 3. Seroprevalence of *T. gondii* among pregnant women based on pregnancy stages

Pregnancy stage	Number of individuals examined	Number of individuals positive	Percentage (%) of individuals positive
1 st trimester	64	16	25.0
2 nd trimester	96	34	35.4
3 rd trimester	32	10	31.3
Total	192	60	31.3

$$X^2 = 15.600, Df = 2, P\text{-value} = 0.00$$

Table 4. Relationship of *T. gondii* antibodies and previous history of miscarriage among sampled pregnant women

History of miscarriage	Number of subjects examined	Number of subjects positive	Percentage (%) of subjects positive	RR
None	149	40	26.8	0.553
Once	33	16	48.5	1.213
Twice	10	4	40	1.278
Total	192	60	31.3	

$$\chi^2 = 33.600, Df = 2, P\text{-value} = 0.00$$

Table 5. Seroprevalence of toxoplasmosis among pregnant women based on educational status

Educational status	Number of subjects examined	Number of subjects positive	Percentage (%) of subjects positive
Primary school	111	42	37.8
Secondary school	36	8	22.2
Tertiary school	21	6	28.6
Others	24	4	16.7
Total	192	60	31.3

$$\chi^2 = 65.333, Df = 3, P\text{-value} = 0.00$$

Table 6. Seroprevalence of toxoplasmosis among pregnant women based on keeping cat

Do you keep cat?	Number of subjects examined	Number of subjects positive	Percentage (%) of subjects positive	RR
Yes	61	14	22.9	0.517
No	131	46	35.1	1.122
Total	192	60	31.3	

$$\chi^2 = 17.067; Df = 1; P\text{-value} = 0.00$$

4. DISCUSSION

This study revealed the presence of IgM to *T. gondii* in pregnant women attending antenatal clinic at some health facilities in Kaduna metropolis and environs. In *T. gondii* infection IgM and IgG are the 2 main immune response exhibited by the host. The presence of IgM detected among the pregnant women sampled is indicative of either a recent *T. gondii* infections or reactivated dormant toxoplasmosis [28].

The overall seroprevalence of 31.3% recorded in this study is relatively higher than that reported by Oyinloye et al. [29] who recorded a prevalence rate of 22.2% in Maiduguri municipal council, Borno State Nigerian in a study of toxoplasmosis carried out among pregnant women. However, Ishaku et al. [28] in a similar study recorded 29.9% prevalence in Zaria, Kaduna State. Relatively lower prevalence of *T. gondii* infection (18.5%) reported by Awoke et al. [30] among pregnant women attending antenatal care at Felege Hiwot Referral Hospital, Northwest Ethiopia despite a higher seroprevalence (83.6%) was earlier reported by Zemene et al. [31] among

pregnant women. Conversely, higher seroprevalence of 63.5% was reported in Colombia [32] and 77.5% in Brazil [33]. Higher prevalence were also reported in some neighbouring African countries from previous studies conducted with prevalence of 63.1% in São Tome and Principe [34], 34% in Sudan [35], 36.3% in Madagascar [36], 37.5% in Somalia [37] and in Ethiopia; 90% from HIV infected and HIV uninfected individuals [38]; 75% from a survey carried out in general population [39]. The detection of antibodies to *T. gondii* in many African countries including Nigeria underscore the need for continuous education of women of child-bearing age on the consequences of the infection on their unborn babies and ways of preventing toxoplasmosis.

The variation in the prevalence of *T. gondii* with age of women tested has been affirmed by many author [40,41,42,43]. The present also demonstrate the effect of age on the prevalence of *T. gondii* infection among antenatal women, with the risk of exposure to *T. gondii* infection increasing with age. In the present study the highest percentage seropositivity (67.7%) was

recorded among the 31-35 yrs age group with a Relative Risk (RR) of 4.620 and lowest percentage positive (17.9%) per age group found among women <20 yr (RR= 0.481). Gebremedhin et al. [44] recorded 81.4% among women of child bearing age. Similarly, to the study of Zemene et al. [31] that observed a significant association between seroprevalence of toxoplasmosis and age of individuals studied. Several intrinsic and extrinsic factors could have been responsible for variation among the different age groups. Some of which may include the level of personal hygiene and socio-economic status of the family, thus highlighting the need to continue to educate women of child-bearing age on prevention of toxoplasmosis.

The relatively high seroprevalence (35.4%) of toxoplasma antibodies found in pregnant women at their second trimester of gestation period in the present study is in agreement with a study in Ethiopia by [45]. The authors recorded the highest seroprevalence of toxoplasma antibodies in pregnant women in their second trimester.

The high seropositivity of 37.8% recorded among pregnant women who had only primary education compared to women with higher education level could be due to difference in knowledge and practice of personal hygiene. This is in agreement with the findings of other authors who reported that lower levels of education were significantly associated with increased risk of toxoplasmosis [28]. This factor explains the variation observed among pregnant women of different occupations sampled. *Toxoplasma gondii* infection is acquired through ingestion of oocyst from contaminated environments in the community, regardless of occupation or educational level although sanitation and personal hygiene plays a significant role in the acquisition of infection.

In previous reports [46,47], regular contact with domestic animals such as cats and their litters were linked with *T. gondii* infection. However, this study observed the seropositivity of toxoplasmosis to be significantly higher (35.1%) among pregnant women that do not keep cats than women who keep cats (22.9%) (P<0.05). This may be because, the mere presence of cat in the house is not enough to confirm zoonosis but rather handling of cats' litter is of more important. The findings in this study are consistence with studies conducted in Ethiopia [28] and Taiwan [29].

5. CONCLUSION

The seroprevalence of recorded toxoplasmosis among pregnant women surveyed in the study area is relatively high compared to similar studies carried out in other parts of Nigeria. The detection of IgM to toxoplasmosis in 31.3% of pregnant women in the study area is indicative of the endemicity of the infection among the populace in the state. Women at the age of 31-35yrs are found to be at higher risk of contracting toxoplasmosis than any other age group. Pregnancy stage (trimester) and history of miscarriage are found to be highly associated with toxoplasmosis.

6. RECOMMENDATIONS

Serological screening for toxoplasma infection should be conducted during antenatal clinics to enable early detection of infection. Public awareness should be embarked upon by relevant government agencies and non-governmental organisation (NGOs) to enlighten the populace on the consequences of toxoplasmosis and the need for prevention.

CONSENT

All authors declared that written informed consent was obtained from the patient (or other approved parties) for publication of this paper and accompanying images.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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