

Seroprevalence and Associated Risk Factors of *Toxoplasma gondii* among Pregnant Women Attending Ante Natal Clinics in Zaria, Kaduna State, Nigeria

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Abstract: Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii* an obligate intracellular parasite. It results in severe complications in the foetus if the infection is acquired during pregnancy including spontaneous abortion, low birth weight, congenital malformations and intrauterine death. The study was Hospital based and cross-sectional and was carried out among pregnant women attending antenatal clinics in Zaria, Kaduna State. The samples were collected from Gambo Sawaba General Hospital Kofan Gayan, Ahmadu Bello University Medical Centre and Major Ibrahim Bello Abdullahi Memorial Hospital. A total of 182 blood samples were collected. Enzyme Linked Immunosorbent Assay (ELISA) method was used to determine the seroprevalence of *Toxoplasma* IgM antibodies. A structured questionnaire was used to collect information on socio-demographic characteristics and factors associated with *Toxoplasma* infection. The study established an overall seroprevalence of 19.2%. There were no significant associations of *T. gondii* infection with all demographic and risk factors measured in this research ($p>0.05$), except ownership of cat (OR=0.23, 95%CI=0.1 – 0.5, $P=0.00$) and blood transfusion (OR=0.04, 95%CI=0.04 – 0.50, $P=0.01$)

Keywords: *T. gondii*, *B1 gene*, Seroprevalence, PCR, Risk factors

INTRODUCTION

Toxoplasma gondii, an obligate intracellular protozoan parasite in the phylum Apicomplexa that is present in a wide variety of animal species worldwide, is the causative agent of the zoonotic parasitic disease known as toxoplasmosis (Tymoshenko *et al.*, 2015, Urquhart *et al.*, 2015). *Toxoplasma gondii* infection is chronic in about 30% of the world's population (Moncada *et al.*, 2012). One of Africa's most common and pervasive parasite illnesses, toxoplasmosis is frequently disregarded, especially in developing nations (Dasa *et al.*, 2021). In immunocompetent people, the infection produced by this organism normally manifests as a cold or the flu and is mild and asymptomatic, although in up to 20% of cases, cervical lymphadenopathy, a condition resembling mononucleosis, or inflammation can occur (Dasa *et al.*, 2021). Encephalitis, pneumonia, and inflammation of the heart muscles can also arise from original or reactivated infection in immunocompromised people, such as AIDS patients (Montoya, 2004).

The only known definitive hosts for this organism are members of the *Felidae* family example domestic cats, making them the primary source of infection. *Toxoplasma*

gondii exclusively sexually reproduces in intestinal epithelial cells in cats, and after a brief period of time (1-2 weeks), oocysts are produced and released into the environment through cat feces (Harold *et al.*, 2004). Infected humans who are unintentional hosts typically do so by accidentally swallowing oocysts from the environment, eating undercooked meat that has tissue cysts, drinking water that has been contaminated with sporulated oocysts, or vertically by transplacental transmission of tachyzoites (Hegab and Al-Mutawa, 2003).

By consuming live tissue cysts in meat or cat oocysts that contaminate the environment, humans might become infected. The majority of human infections are asymptomatic, however the parasite can cause fatal diseases. Only 10% of infected people experience signs and symptoms. Oocyst-induced infections are more severe than those brought on by tissue cysts (Saad *et al.*, 2012).

The most common clinical manifestation of toxoplasmosis in humans is enlargement of the lymph nodes, which is accompanied by fever, exhaustion, muscle discomfort, sore throat, and headache. Despite the possibility that the illness is benign, pregnant women must be diagnosed because of the potential risk to the fetus (Si *et al.*, 2009).

The aim of this research was to determine the Seroprevalence of *Toxoplasma gondii* among Pregnant Women Attending Antenatal Clinics in Zaria, Kaduna State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out in Zaria, a major city in Kaduna State, Nigeria. It has a total area of 563km², a density of 730/km². It is located on a plateau at a height of 2,200feet above sea level with a projected population of 766,000 (NPC, 2023). Zaria is a big city in Kaduna state with people living together in peace and harmony, and is blessed with farmlands where people farm during rainy season and also dry season, the people are also known for farming vegetables such as tomatoes, cabbage, onions, carrots. Women also sell those vegetables and, in the process, eat them without proper washing or cooking. There are many suya joints around where people buy stick meat with some not properly handled. Most houses keep cats around as pets and cats are one of the major reservoirs for the parasite *T. gondii*.

Study Design

A Convenience sampling method was used in the selection of the study population from Ahmadu Bello University Medical Centre, Gambo Sawaba General Hospital Kofan Gayan, and Major Ibrahim Bello Abdullahi Hospital Zaria, Nigeria. The study was hospital based and cross-sectional.

Study Population

The study population was pregnant women attending antenatal care at Ahmadu Bello University Medical Centre, Gambo Sawaba General Hospital Kofan Gayan, and Major Ibrahim Bello Abdullahi Hospital Zaria, Nigeria.

Inclusion criteria

Pregnant women attending antenatal care on their first booking in the three health facilities and who gave consent were included in the study.

Exclusion criteria

Pregnant women who did not consent or did not register with the clinics and those on

subsequent visit were excluded during the study.

Ethical Approval

The Ethical Approval was obtained from the Research and Ethics Committee of the Ministry of Health, Kaduna State with a reference number of MOH/ADM/744/VOC/919

Sample Size

A sample size of 182 was obtained using a previous prevalence of 8.9% by Idris *et al.* (2015). The total sample size was estimated using the standard formula (Lwanga and Lemeshow, 1991)

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

Where n = sample size,

Z = Z statistic for a level of confidence,

P = expected prevalence or proportion (8.9%)

d = precision (in proportion of one; if 5%, d = 0.05).

$$N = (1.96)^2 \times 0.89(1-0.89) / (0.05)^2$$

$$= 3.8416 \times 0.11 / 0.0025$$

$$= 150.437056$$

$$= 150$$

Sample size = 150 samples.

For more precision and accuracy, 182 samples were collected for the study, 60 samples were collected from Ahmadu Bello University Medical Centre and Major Ibrahim Bello Abdullahi Memorial Hospital respectively while 62 samples were collected from Gambo Sawaba General Hospital

Questionnaire administration

Structured questionnaires were used to obtain data on demographic and risk factors such as age, history of miscarriage, trimester, presence of cat and source of drinking water. Volunteers were adequately educated on how to complete the questionnaire and others were assisted to complete theirs on collection of their specimen.

Sample Collection and Processing: Blood samples (5mls) were collected by aseptic venipuncture procedure in sterile plain containers from the pregnant women under study.

Samples collected were transported in cold packs to the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria. The blood samples were centrifuged at 1000rpm for 3minutes to separate serum and stored in refrigerator at -20°C for further analysis.

Detection of *Toxoplasma* IgM by ELISA

All procedures were carried out according to the manufacturer's instruction (Vircell Microbiologists). Samples and reagent under refrigeration were brought to room temperature (24°C). Pre-coated microplates were duly labeled (Blank, positive, negative controls and test serum samples). Hundred microlitre (100µl) of sample dilution solution (100µl) was placed in the wells of microtitre plates using a micropipette except those assigned for controls. Five (5 UI) of the samples, hundred (100 µl) of positive control, hundred (100 µl) of cut off solution (in duplicate) and hundred (100 µl) of negative control were added into each wells. The plates were then covered with a sealing sheet and incubated at 37°C for 60minutes.

Antibody Index

Index	Interpretation
< 9	Negative
9 – 11	Equivocal
>11	Positive

Data Analysis

The data obtained from the questionnaire and the result of the laboratory analysis were entered into Microsoft excel and analysed using Statistical Package for Social Science version 20. The Pearson Chi-square test at 95% confidence interval and 0.05 level of significance was used to determine the relationship between demographic data and prevalence.

RESULTS

Out of 182 blood samples screened for anti-*Toxoplasma gondii* IgM using ELISA, thirty-five (35) samples were positive with a prevalence of 19.2%. The result is shown in Figure 1.

After the incubation, the seal was removed and all the liquid in the wells were aspirated and then the wells were washed five times with 0.3ml of washing solution per well. Immediately 100 µl of reconstituted conjugate was added into each well, sealed with a sealing sheet and incubated at 37°C for another 60minutes. After incubation, 0.3ml of washing buffer was added into each well and washed five times. After washing 100 µl of substrate solution was added into each well and incubated at room temperature for 20minutes protected from light after which 50 µl of stopping solution was added into each well and read with a spectrophotometer at 450/620nm within 1hour of stopping.

Interpretation of ELISA Result

The mean optical density was obtained, the value obtained should not be > 0.55 or < 1.55 as instructed by the manufacturer (Vircell Microbiologists). Antibody index was calculated using sample optical density / cut off serum optical density × 10, based on the manufacturer's instruction.

Table 1 shows seroprevalence of *Toxoplasma gondii* among pregnant women attending ante natal clinics based on the sampling sites. Gambo Sawaba General Hospital (GSGH) was found to have the highest prevalence of 14.3%, followed by Major Ibrahim Bello Abdullahi Memorial Hospital (MIBA) with a prevalence of 2.75% while the Ahmadu Bello University Medical Centre (ABUMC) had the lowest value of 2.20%.

Table 2 shows seroprevalence of *T. gondii* among pregnant women attending ante natal clinics in relation to socio demographic factors. The distribution of the *T. gondii* infection in our study revealed that of the 182 respondents, the age group 15 – 19years had the highest prevalence of 4.4%.

The least prevalence of 0% was observed in the age group 40 – 49years. All the participants were married women and had a prevalence of 19.2%. The distribution according to residency showed rural dwellers sero-prevalence of 1.1% as against 18.1% for the urban dwellers. Women with secondary education showed the highest prevalence of 13.7%, and the least of zero prevalence was recorded in those with tertiary education. Participants who had other forms of employment showed a higher prevalence of 19.2% and zero prevalence was recorded for unemployed.

Table 3 shows seroprevalence of *T. gondii* in relation to risk factors in pregnant women attending antenatal clinics in Zaria, Kaduna State. Based on the study all the pregnant women eat meat and had a prevalence of 19.2%, those that fully cooked their meat had higher prevalence of 17.0% and lower prevalence in those that boil and eat their meat half cooked 2.2%. Participants that boiled their meats had the highest prevalence of 13.8% and the lowest prevalence of 0.5% was recorded in those that roasted. Almost all the pregnant women in the study eat stick meat and had a prevalence of 19.2%.

According to the study women that drink well water had the highest prevalence of 17.6% and the lowest prevalence (0%) was recorded in women that drink packaged water. All the women under study eat vegetables and a prevalence of 19.2% was recorded, those who eat raw vegetables had the highest prevalence of 18.6% while those that steam their vegetables had the lowest prevalence 0%. Pregnant women who own cats had higher prevalence of 12.1% and lower prevalence of 7.1% was recorded in those that don't own cats.

Table 4 shows Seroprevalence of *T. gondii* among pregnant women attending ante natal clinics in Zaria, Kaduna State in relation to clinical information. The highest prevalence (15%) was found in women who were in their second trimester of pregnancy and lowest prevalence of 0.5% was recorded in women that were in their first trimester. Pregnant women who did not receive blood transfusion had a higher prevalence of 17.6% as compared to pregnant women that were transfused 1.6%. Multigravida had a higher prevalence of 48.9% and a lower prevalence of 6.0% was recorded in the primigravida.

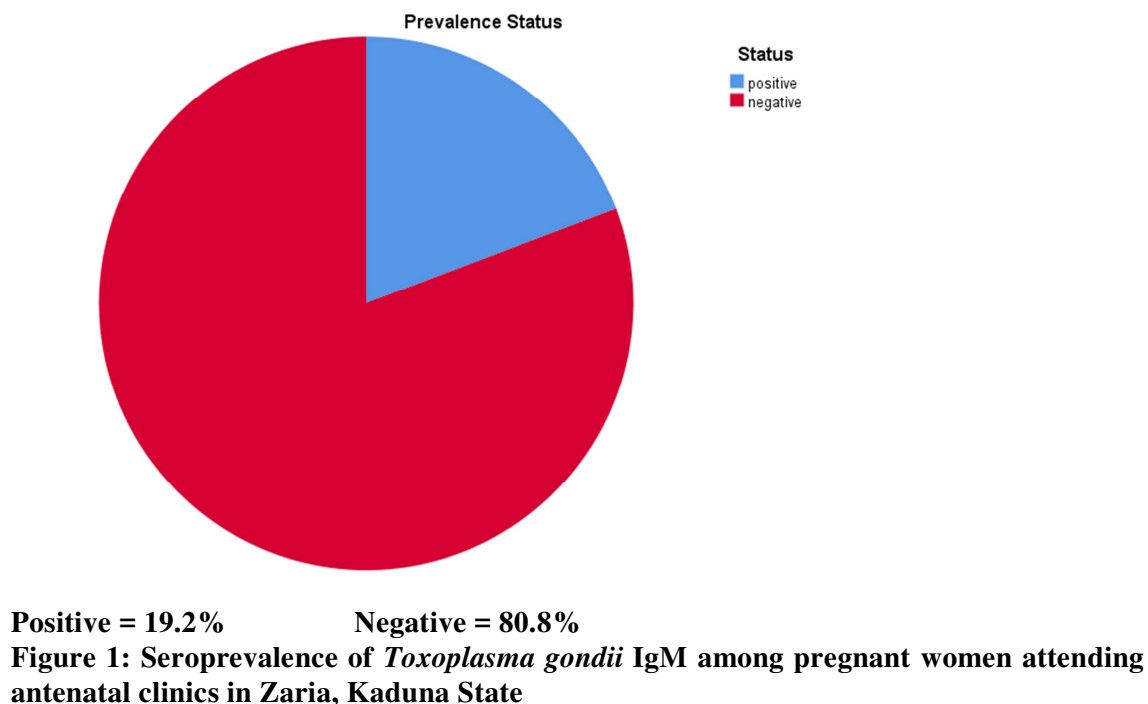


Table 1: Seroprevalence of *T. gondii* among pregnant women attending antenatal clinics in Zaria, Kaduna State, in relation to sampling site

Study site	Number examined	Positive (%)	Chi square	ρ value
ABUMC	60	4(2.2)	19.72	0.00
MIBA	60	5(2.7)		
GSGH	62	26 (14.3)		
Total	182	35(19.2)		

Key: ABUMC: Ahmadu Bello University Medical Centre, MIBA: Major Ibrahim Bello Abdullahi Memorial Hospital, GSGH: Gambo Sawaba General Hospital

Table 2: Seroprevalence of *T. gondii* among pregnant women attending ante natal clinic in Zaria, Kaduna State in relation to socio-demographic factors

Variable	Number examined	Positives (%)	chi square	ρ value	OR	CI
Age group(years)						
15 – 19	28	8(4.4)	6.99	0.69	0.82	0.36 - 2.23
20 – 24	46	7(3.8)				
25- 29	35	4(2.2)				
30 – 34	38	9(4.9)				
35 – 39	28	7(3.8)				
40 – 49	7	0				
Marital status						
Married	182	35(19.2)	0	0	0	0 – 0
Unmarried	0	0				
Residence						
Urban	172	33(18.1)	0.04	0.99	1.01	0.19 - 5.40
Rural	10	2(1.1)				
Level of education						
None	13	1(0.5)	2.84	0.7	0.89	0.49 - 1.63
Primary	32	9(5.0)				
Secondary	132	25(13.7)				
Tertiary	5	0				
Occupation						
Unemployed	1	0	0.36	0.57	1.79	0.24 - 13.71
self employed	7	1				
Others	174	35(19.2)				
Disease knowledge						
Yes	0	0	0.00	0	0	0
no	182	182(100)				

Table 3: Seroprevalence of *T.gondii* in relation to risk factors in pregnant women attending antenatal clinics in Zaria, Kaduna State

Variable	Number examined	Positives (%)	chi square	ρ value	OR	CI
Eating of meat						
Yes	182	35 (19.2)	0	0.00	0	0
No	0	0				
Form of meat						
Cooked	160	31 (17.0)	0.02	0.96	0.97	0.29 - 3.13
Boiled	22	4 (2.2)				
Processing method						
Cooked	2	0	1.2	0.71	0.87	0.41 - 1.85
Fried	37	9 (4.9)				
Boiled	137	25 (13.8)				
Roasted	6	1(0.5)				
Tasting while cooking						
Yes	182	35(19.2)	0	0.00	0	0
No	0	0				
Eating of stick meat						
Yes	181	35(19.2)	0.24	1	0	0
No	1	0				
Water type						
Packaged	2	0	0.74	0.49	1.54	0.44 - 5.39
Well	167	32(17.6)				
Piped	13	3(1.6)				
Eating vegetables						
Yes	182	35(19.2)	0	0.00	0	0
No	0	0				
Form of eating vegetables						
Raw	76	34(18.7)	0.56	0.72	0.73	0.12 - 4.31
Cooked	4	1(0.5)				
Steamed	2	0				
Owning of cat						
Yes	65	22(12.1)	13.26	0.00	0.22	0.09 - 0.49
No	117	13(7.1)				

Table 4: Seroprevalence of *T. gondii* in relation to clinical information among pregnant women attending ante natal clinics in Zaria, Kaduna State

Variable	Number of examined	Positives (%)	chi square	ρ value	OR	CI
Gravidity						
primigravida	20	11(6.0)	0.67	0.5	0.69	0.23 - 2.06
multigravida	162	89(48.9)				
Trimester						
first	3	1(0.5)	0.52	0.86	0.92	0.34 - 2.50
second	147	28(15.3)				
third	32	6(3.4)				
Blood transfusion						
Yes	4	3(1.6)	8.19	0.01	1	0.52 - 1.95
No	178	32(17.6)				

DISCUSSION

An overall prevalence of 19.2% was recorded in the study. This is lower than the reports of other studies within Kaduna State. Ishiaku *et al.* (2009) reported an overall prevalence of 29.9% and Bello *et al.* (2017) reported an overall prevalence of 30.3%. These differences may be as a result of differences in sampling sites even within the same State. The study is also lower when compared to other studies conducted within Nigeria, 22.2% from Maiduguri (Oyinloye *et al.*, 2014) and 40.8% in Lagos (Akinbami *et al.*, 2010). The differences in seroprevalence may be as a result of variation in geographical location, temperature and wind even within the same country of different regions as the oocyst sporulation is enhanced by hot and wet conditions (Tenter *et al.*, 2000, Logar *et al.*, 2002).

The occurrence, survival, distribution, and transmission of *T. gondii* are influenced by climate factors primarily in three ways.

The local climate, notably the temperature and humidity, has an impact on whether or not oocysts can sporulate (Patz *et al.*, 2000). Seasonal precipitation has an impact on river flow, which in turn impacts how easily oocysts can travel from the land to water, including the ocean. This can result in water-borne toxoplasmosis and high levels of coastal contamination with this protozoan parasite (Karanis *et al.*, 2013; Mazzillo *et al.*, 2013; Ribeiro *et al.*, 2013).

The geographic distribution, population density, and migration patterns of rodents, migratory birds, and arthropods, animals that serve as reservoir hosts or transport hosts and are crucial to the emergence, survival, spread, and transmission of *T. gondii* could also be impacted by the climate (Root *et al.*, 2003; Afonso *et al.*, 2013)

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According to the survey, Ahmadu Bello University Medical Center had the lowest prevalence (2.2%) and Gambo Sawaba Hospital had the highest prevalence (14.3%). The research population's differences could be the cause of the variance in prevalence. The pregnant patients at Gambo Sawaba General Hospital are more susceptible to the infection due to factors like not having access to potable water, owning cats, and improper handling of with vegetables because some of them work in the vegetable markets. A significant association was found between ownership of cat and toxoplasmosis. Cats are the definitive hosts of this organism in which sexual reproduction take place resulting in oocyst that are passed out in the cats faeces, although cats are secretive about defecating they clean up themselves using their paws and rub all over their furs, human come in contact with it and get infected in the process. This is similar to the finding of Dairo *et al.* (2018).

A significant association was recorded between *T. gondii* infection and blood transfusion. This agrees with a study in Nigeria (Dairo *et al.*, 2018) and in Rwanda (Esperance *et al.*, 2017). The pregnant women may have acquired the infection through the transfused blood from infected donor. This is supported by (Abu Madi *et al.*, 2010) in their study.

CONCLUSION

This study established 19.2% sero prevalence of *T. gondii* among pregnant women attending ante natal clinics in Zaria. There was no significant association of *T. gondii* infection with all demographic and risk factors measured in this research ($p > 0.05$), except ownership of cat and blood transfusion ($p < 0.05$).

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