ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 7 No. 1 March 2021*

Seroprevalence of *Toxoplasma Gondii* Antibodies Amongst Neonates in Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria, Nigeria

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Abstract

Toxoplasma gondii is an obligate intracellular protozoan parasite, which causes toxoplasmosis with a global distribution amongst humans and animals. Congenital toxoplasmosis generally occurs when a woman is newly infected with T. gondii during pregnancy and transfer the parasite unknowingly to the foetus. This study was carried out to determine the seroprevalence of T. gondii among neonates in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. Prior to sample collection, well-structured pre-tested questionnaires were used to obtain socio-demographic data and possible risk factors of the neonates through their biological mothers. A total of 92 blood samples were collected for the screening of T. gondii antibodies from neonates in this study. Due to the ages of the neonates and the condition they were born into, some of the blood samples (n = 4) collected were not up to the quantity required for screening of both antibodies. As such, only IgM were screened for such samples leaving out IgG (i.e. IgM, n = 92; and IgG, n = 88). It was found that 23.9% (21/88) and 2.2% (2/92) neonates were positive for IgG and IgM antibodies respectively. Higher prevalence of T. gondii IgG was found among females (30.4%: 14/46) (χ^2 = 0.627, df = 4, P = 2.597) compared to males (16.7%:7/42). T. gondii IgG antibodies were detected with the highest prevalence among neonates of 16-20 days (33.3%:7/21) while the lowest was recorded in neonates of 6-10days (14.3%:2/14). It was concluded that toxoplasmosis among neonates in ABUTH is not common and detection for IgG and IgM antibodies before or during pregnancy is recommended.

Keywords: *Toxoplasma gondii,* Antibodies, Neonates, Congenital, ELISA, IgG, and IgM

Introduction

Toxoplasma gondii is the causative agent of toxoplasmosis (Oyeyemi *et al*., 2019). *T. gondii* is a parasitic protozoan with an obligate intracellular mode of living (Gyang *et al*., 2015). It can reproduce asexually in varying tissues of their intermediate hosts being mostly vertebrates. However, *T. gondii* reproduce sexually in epithelial tissues of the digestive tracts of cats

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being the definitive hosts. Cats get infected with *T. gondii* when they ingest meats contaminated by the cyst but rarely infected through direct ingestion of oocysts disseminated by feces of other infected cats (Nasir *et al*., 2015; Sharif *et al*., 2018). *T. gondii* is a zoonotic pathogen of warm-blooded animals including birds and man (Bello *et al*., 2017). *T. gondii* is however, transmitted horizontally to humans through consumption of water, fruits, vegetables, food and undercooked meat that has been contaminated with oocysts of *T. gondii* (Farouk *et al*., 2020; Fanigliulo *et al*., 2020). *T. gondii* has an incubation period of 5-18 days following infection of a susceptible host (Nasir *et al*., 2015), it can however remain inactive and persist all through the life time of the infected persons (Ohiolei and Isaac, 2016).

Over 70% of the world population is infected with *T. gondii*. Although the prevalence of this parasite varies from one country to another (Fanigliulo *et al*., 2020), however, *T. gondii* has been reported to be present in every country of the world (Karshima and Karshima, 2020). Immunocompetent individuals infected with *T. gondii* are often asymptomatic and show little or no clinical symptoms. Some of the symptoms caused by toxoplasmosis in immunocompetent individuals include fatigue, listlessness, excessive sweating, headache, joints and muscle pains, retinochoroiditis, fever and maculopapular rash (Ohiolei and Isaac, 2016; Karshima and Karshima, 2020). Due to occurrence of similar symptoms of toxoplasmosis with mononucleosis or dromes makes acute toxoplasmosis to be undiagnosed as such, it is mostly not reported (Ohiolei and Isaac, 2016). However, individuals who show cervical lymphadenopathy with other forgoing symptoms are often suspected to be suffering from toxoplasmosis. Clinical manifestation of toxoplasmosis is common among individuals below 15 years alongside other immunocompromised persons (Ohiolei and Isaac, 2016).

Although *T. gondii* affects all age group across the world, it is more deleterious to pregnant women and their neonates (Oyeyemi *et al*., 2019). Pregnant women who are infected with *T. gondii* can be haematogenously transferred from the mother to the placenta and infects the foetus in a process known as vertical transmission to cause what is known as congenital toxoplasmosis (Oyeyemi *et al*., 2019). The extent of health defects caused by congenital toxoplasmosis depends on the gestation stage of the pregnancy and the length of exposure (Fanigliulo *et al*., 2020). However, the general risks caused by untreated congenital toxoplasmosis ranges from 20-50% (Fanigliulo *et al*., 2020). Some of the health defects caused by congenital toxoplasmosis include hydrocephaly or microcephaly, cerebral calcifications, miscarriage, still birth, or abortion (Ohiolei and Isaac, 2016; Fanigliulo *et al*., 2020; Karshima and Karshima, 2020). Children that survive may experience developmental problems, including attention deficit, mental retardation, ocular dysfunction (chorioretinitis), lymphadenopathy, hepatosplenomegaly, seizures, epilepsy and even schizophrenia (Oyinloye *et al*., 2014; Ohiolei and Isaac, 2016; Adeniyi *et al*., 2018; Orevaoghene and Wogu, 2020).

In order to prevent health impacts caused by congenital toxoplasmosis, early detection of IgM and IgG antibodies of *T. gondii* is paramount among both infected and non-infected pregnant women as well as providing vital information on how to prevent contracting the parasite as well as serological follow up of those who have tested positive to the parasite (Nasir *et al*., 2015). Developed nations such as Germany, France and Austria have been able to reduce the occurrence of congenital toxoplasmosis through compulsory testing of pregnant women going for antenatal (Adeniyi *et al*., 2018). However, low awareness in developing countries and cost of tests, reagents and equipment has deterred early detection of *T. gondii* among pregnant women thereby increasing its prevalence. Karshima and

Karshima (2020) reported continental prevalence range of *T. gondii* to be 6.8–51.8% in Europe, 10.6–13.0% in North America, 14.0–96.3% in Asia, 26.3–80.0% in South America, and 4.3–75.0% in Africa (Karshima and Karshima, 2020). These values however, vary between individual nations due to different hygiene practices, dietary habits and socioeconomic status of its citizens (Fanigliulo *et al*., 2020).

In Nigeria, the seroprevalence of *T. gondii* also varies across states. Oyinloye *et al*. (2014) reported a prevalence of 40.8% in Lagos, 44.4% in Calabar, 21.6% in Sokoto, and 22.2% in Maiduguri. Adeniyi *et al*. (2018) reported a prevalence of 30.44% in Osogbo; Farouk *et al*. (2020) reported a prevalence of 20.5% in Gombe. Low awareness and knowledge of *T. gondii* is lacking in Nigeria as studies carried out by Adeniyi *et al*. (2018) reported that 90.4% of women assessed were not aware of the existence of *T. gondii* neither were they aware of the health impact caused by it. As such this study was aimed at determining the seroprevalence of *T. gondii* among pregnant women attending antenatal in Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, Nigeria.

Materials and Methods

Study Area

The study was hospital based and was conducted at Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria, Nigeria. Zaria, Nigeria is located at latitude 11.085541, and the longitude is 7.719945 with coordinates 11° 5′ 7. 9476″ N and 7° 45′ 11. 8020″ E respectively. The hospital is positioned in Shika, a small settlement about 2km away from Samaru, Sabon Gari Local Government Area Kaduna, State.

Study Population

The target population was some neonates (babies at the first 28days of birth), in the special baby care unit of the ABUTH Zaria.

Inclusion Criteria

Babies of 28 days old and below.

Exclusion Criteria

Babies more than 28 days old.

Sample Size Determination

The sample size was determined using the formula by Agabi *et al* (2010);

$$n = \frac{Z^2 \times P \times q}{D^2}$$

Z = confidence level (0.95); P = prevalence 87% = 0.87; D = sample error (0.05);
q = 1-p =1-0.87 = 0.13
Therefore $n = \frac{0.95^2 \times 0.87 \times 0.13}{0.05^2} = 40$

The calculated sample size was 40 hence; the least number of samples that could be used for the study was 40.

Ethical Approval
Permission and approval for the study was obtained from the medical board of the Ahmadu Bello University Teaching Hospital (ABUTH) Zaria with an introductory letter signed by the Head of Department, Microbiology Department, Ahmadu Bello University Zaria.
The aim of the study was clearly explained to the mothers of the neonates and informed consent was obtained before administering questionnaires. To ensure confidentiality, names of the respondents were not recorded on the questionnaire. The questionnaire was interpreted in local languages for those who do not understand English.

Research Tool Administration
Prior to sample collection, demographic data such as sex, contact with cats, contact with soil, drinking of unpasteurized milk, from the mothers of the neonates using a structural questionnaire were obtained.

Sample Collection and Processing
A total of 92 blood samples were collected for the screening of *T. gondii* antibodies from neonates in this study. Due to the ages of the neonates and the condition they were borne into, some of the blood samples (n = 4) collected were not up to the quantity required for screening of both antibodies. As such, only IgM were screened for such samples leaving out IgG (i.e. IgM, n = 92; and IgG, n = 88). Prior to sample collection, socio-demographic data such as sex, age of baby, ownership of cat, eating raw or undercooked meat, contact with soil, drinking of unpasteurized milk, eating of unwashed fruits and vegetables were all captured. About 2 mL of the blood samples were collected in a sterile plain bottle and transported back to the Department of Microbiology Ahmadu Bello University, Zaria in an ice bag. The samples were allowed to thaw then centrifuged at 1500 rpm for about 15 min. Using a sterile Pasteur pipette, the sera was separated and labelled accordingly before storing it in a freezer at -20°C till further use.

Sample Analysis
The samples were analysed using the commercial immunoassay kit Diagnostic Automation In-cooperation for *Toxoplasma* IgG and IgM analysis of all immunoassay procedures were performed according to the manufacturers' instructions.

Assay Procedure
The blood samples and all the reagents were brought to room temperature (18-32°C); the stock wash buffer was diluted 1 bottle to 100 mL of distilled water. The micro wells were numbered, including 2 for negative control, 1 for positive control, and 1 for blank. A 1:40 dilutions was prepared by adding 5 µL to the test samples, negative control, positive control, and calibrators to 200 µL of sample diluent and was mixed well. A 100 µL of diluent sera, calibrators, and controls were dispensed into the appropriate wells. For the blank reagent, 100 µL sample diluent was dispensed in 1A well position. The holder was tapped to remove air bubbles from the liquid and mixed well. It was then incubated for 30 min at room temperature. Liquid was removed from all the wells and washed three times using the washing buffer. A 100 µL of enzyme conjugate was dispensed to each well and incubated for 30 mins at room temperature. The enzyme conjugate was removed from all the wells and washing was repeated three times with washing buffer. A 100 µL of chromogenic substrate was dispensed to each well and incubated for 15 mins at room temperature then 100 µL of the stop solution was added. The result was read immediately at 450 nm with a microwell reader.

Results

Seroprevalence of IgG and IgM antibodies to T. gondii among neonates in ABUTH, Zaria

The results of this study revealed the presence of *T. gondii* IgG antibodies in 21 new-borns (23.9%) and IgM in 2 new-borns (2.2%) as shown in Table 1.

Table 1: Seroprevalence of *T. gondii* antibodies among neonates

Antibody type	Total Sample Screened	No. positive	Percentage (%)
IgG	88	21	23.9
IgM	92	2	2.2

Seroprevalence of T. gondii in relation to age group and sex of neonates

The sero prevalence of *T. gondii* according to age (Table 2) showed highest prevalence of IgG antibodies (33.3%:7/21) among babies who were 16-20 days and the lowest (14.3%:2/14) among those who were 6-10 days (p = 0.5533). Also, IgM antibodies were detected in only two of the neonates who were in the age group 6-10 days and 11-15 days (p = 0.64).

Out of the 88 samples screened, 7 males (16.7%:7/42) and 14 females (31.1%: 14/46) tested positive to *T gondii* IgG antibody. Whereas 2 neonates; 1 male (2.2%:1/45) and 1 female (2.2%:1/45) tested positive to *T. gondii* IgM antibody with *P*-value = 0.987 as shown in Table 2.

Table 2: Seroprevalence of *T. gondii* in relation to age group and sex of neonates

		** IgG			* IgM		
Variable	Total	No Positive	Percentage	Total	No positive	Total	P-value
Age group (days)							
0-5	19	4	21.1	20	0	0.0	*=0.64
6-10	14	2	14.3	16	1	6.3	**=0.5533
11-15	21	6	28.6	22	1	4.5	
16-20	21	7	33.3	21	0	0.0	
21-25	13	2	15	13	0	0.0	
Sex							
Male	42	7	16.7	45	1	2.2	*116
Female	46	14	30.4	47	1	2.2	**0.987

Seroprevalence of T. gondii according to clinical manifestation

Out of 17 neonates who had symptoms of fever, only 8 (47.1%) tested positive to IgG antibodies while 1 (5.6%) out of 18 neonates that had symptoms of fever tested positive to IgM antibodies. There was significant difference p = 0.015 with patients tested for IgM antibodies. However, no significant difference was seen for IgM antibodies p = 0.307 among neonates who had fever as shown in Table 3.

Table 3: Seroprevalence of *T. gondii* according to clinical manifestation

Variables	No of Samples	No of Positive	No of Negative	P-value
IgG				
Fever				
Yes	17	8(47.1)	9(52.9)	0.015
No	65	12(18.5)	53(81.84)	
NA	06	0 (0.0)	6(100)	
IgM				
Fever				
Yes	18	1(5.6)	17(94.4)	0.307
No	68	1(1.5)	67(98.5)	
NA	06	0 (0.0)	6(100)	

NA= Not Available

Seroprevalence of T. gondii according to knowledge, source of knowledge and clinical manifestation

The result was analyzed according to those with the knowledge of *T. gondii*, and (42.9%: 3/7) which had knowledge of the parasites had antibodies. However, of those with no knowledge, (22.2%: 18/81) also had antibodies to *T. gondii*. The difference in sero-Prevalence obtained was not statistically significant (χ^2= 1.510, df = 1, p = 0.219). Further analysis according to those who had knowledge in relation to the source shows that a marginal difference (P=0.053) exists. All those who got the knowledge from the hospital had antibodies while only one out of five who got knowledge from media had antibodies to *T. gondii* as shown in Table 4.

Table 4: Seroprevalence of *T. gondii* according to knowledge, source of knowledge and clinical manifestation

Variables	No. of samples (%)	No. of positive (%)	No. of negative (%)
IgG			
Knowledge			
Yes	7 (7.95)	3(42.9)	4(57.1)
No	81(92.05)	18(22.2)	63(77.8)
IgM			
Knowledge			
Yes	8(8.7)	1(12.5)	7(87.5)
No	84(91.3)	1(1.2)	83(98.8)
IgG			
Where			
Hospital	2(2.17)	2(100.0)	P = 0.053
Media	6(6.52)	1(20.0)	
IgM			
Where			
Hospital	2(2.17)	0(0.0)	P = 0.537
Media	6(6.52)	1(16.7)	

Seroprevalence of Toxoplasma gondii in relation to various risk factors (IgG)

The result was analysed according to the risk factors that might be associated with *T. gondii* is shown in Table 4. In relation to ownership of cat, those that had cats had higher

prevalence of (22.2%:4/18) and lowest prevalence compared to those who do not own cats (24.3%: 17/70). The difference observed in the seroprevalence was not statistically significant ($\chi^2 = 0.034$, df = 1, p = 0.855).

Analysis of the result in relation to eating raw or undercooked meat showed a higher prevalence of (33.3%:2/6) compared to those that do not eat raw or undercooked meat (23.2%:19/82). The difference observed in seroprevalence was not significant ($\chi^2 = 0.318$, df = 1, p = 0.573).

In relation to contact with soil *T. gondii* antibodies was detected with a higher prevalence of (33.3%:4/12) compared to those that do not have contact with soil (22.4%:17/76). The difference observed in seroprevalence was not significant ($\chi^2 = 0.686$, df = 1, p = 0.408).
In relation to Drinking unpasteurized milk, a significant prevalence of (47.1%:8/17) was obtained among those who drank unpasteurized milk compared to those who do not drink unpasteurised milk (18.3%:13/71) ($\chi^2 = 6.239$, df = 1, p = 0.012).

In relation to eating unwashed fruits and vegetables higher prevalence of (28.6%:2/7) was obtained compared to those who do not eat unwashed fruits and vegetables (23.5%:19/81) for those that do not eat unwashed fruits and vegetables. The difference observed in seroprevalence was not significant ($\chi^2 = 0.093$, df = 1, p = 0.761).
Analysis of the result in relation to the presences or absence of fever in mothers of the neonates showed a significant difference in the prevalence obtained ($\chi^2 = 5.976$, df =1, P= 0.015). Whoever there was no significant difference ($\chi^2 = 1.046$, df =1, p = 0.307) between the prevalence of IgM antibodies and presence of fever. The clinical manifestations presented by the patients presenting with fever 1(5.6%) and those without fever was 1(1.5%). The difference in seroprevalence obtained was however not statistically significant ($\chi^2 = 1.046$, df =1, p = 0.307) for IgM as shown in Table 5**.**

Table 5: Seroprevalence of *Toxoplasma gondii* in relation to risk factors (IgG)

Variable	No of sample	No positive (%)	No of Negative	P-value
IgG				
Own a cat				
Yes	18	4(22.2)	14(77.78)	0.855
No	70	17(24.3)	53(75.71)	
Raw or undercooked meat				
Yes	6	2(33.3)	4(66.67)	0.573
No	82	19(23.2)	63(76.83)	
Contact with soil				
Yes	12	4(33.3)	8(66.67)	0.408
No	76	17(22.4)	59(22.37)	
Consumption of unpasteurized milk				
Yes	17	8(47.1)	9(52.94)	0.012
No	71	13(18.3)	58(81.69)	
Consumption of unwashed fruits and vegetable				
Yes	7	2(28.6)	5(71.43)	0.761
No	81	19(23.5)	62(76.54)	

Discussion

Antibodies are important in detection of so many infections relating to the blood and its component; they are often produced in response to the presence of antigens or any infectious agents in the body. Like every other infectious agent, *T. gondii* causes an immune response by the body through the production of antibodies (IgG and IgM) responsible to fight this parasite (Bello *et al*., 2017). As such, the *T. gondii* IgG and IgM specific antibodies are used for the diagnosis of toxoplasmosis (Bello *et al*., 2017). Result of the present study showed seropositivity of *T. gondii* to be 23.86% (21/88) for IgG and 2.17% (2/92) for IgM antibodies respectively. The seroprevalence of specific antibodies recorded in this study (23.86% for IgG and 2.17% for IgM) agree with the result obtained by Orevaoghene and Wogu (2020), which recorded seroprevalence of *T. gondii* specific antibody IgG to be 32% while IgM was 2%. An overall seroprevalence of 25% (IgG and IgM) antibody was recorded in this study and was quite similar to the 22.2% obtained by Oyinloye *et al*. (2014) and lower than 31.3% obtained by Bello *et al*. (2017). These dissimilarities could be as result of difference in the hygiene practices relating to the mothers of test subjects, sensitivity of test kits, types of subjects (i.e. pregnant and non-pregnant women, neonates, children ≤ 15 years, and adults) as well as varying geographical dispensation.

The seroprevalence of *T. gondii* specific antibodies in relation to age was found to be highest (33.3%) among children aged 16-20 days. Though study of Uttah *et al*. (2013), Gyang *et al*. (2015) and Fanigliulo *et al*. (2020) reported an age-related increase in the seroprevalence of *T. gondii* specific antibody. However, this study saw a scattered pattern of prevalence rate among varying age group, which is in concordance with the result reported by Oyinloye *et al*. (2014), Imam *et al*. (2016), and Adeniyi *et al*. (2018) where there was no consistent pattern of seroprevalence increase in association with the ages of test subjects.

There is a high development of innate immunity and other immune responses in females. However, humans regardless of their sexes are prone to infections provided that all necessary health precautions are not followed as well as other underlining health disorder (Kennedy *et al*., 2014; Ruggieri *et al*., 2016). The overall seroprevalence of *T. gondii* specific antibodies in relation to sexes is most prevalent (31.91%) in females than males (17.8%) in this study. This could be as a result of high immune response to antigens exhibited generally by females making them highly detectable (Ruggieri *et al*., 2016). However, this result differs from the one obtained by Orevaoghene and Wogu (2020), which reported high seroprevalence rate among males (42.12%) compared to females (36.89%). Reasons could be as a result of infectious rate of the parasite or the hygiene practices observed by the mothers of these neonates. Most studies on toxoplasmosis including Oyinloye *et al*. (2014), Imam *et al*. (2016), Bello *et al*. (2017), Adeniyi *et al*. (2018) among many others have been focussed on pregnant women and females within the child bearing age because of the adverse effects it causes to their pregnancy and children that survive the gestation period. As such, there is paucity of data on the seroprevalence of *T. gondii* specific antibodies in relation to sexes of neonates in Nigeria.

Clinical manifestations are often used in diagnosis of several health disorders; some may show signs and symptoms early others do not show until the diseases reach a later stage causing severe damage to host cells if adequate measure is not implemented. Like other diseases, toxoplasmosis causes fever, though most of its symptoms are often confused for flu or cold due to their similarities as it has been seen in this study where 47.1% of neonates who had fever tested positive for IgG while 5.6% tested positive for IgM. This is in line with

the reports of Ohiolei and Isaac (2016), which stated that toxoplasmosis has similar symptoms with flu and cold.

Toxoplasmosis is a neglected disease in Nigeria since adequate awareness is not raised among its citizens predisposing individuals to this parasite (Ohiolei and Isaac, 2016; Oyeyemi *et al*., 2019). The inadequate knowledge of *T. gondii* was confirmed in this study as record 92.05% of the participants tested for IgG and 91.3% were ignorant of *T. gondii* infections and the effects its effects on their babies. The few participants (7.95% for IgG and 8.7% for IgM) reported to have knowledge about toxoplasmosis came through media publicity. This implies that, health workers in hospitals and clinics have little or no knowledge of toxoplasmosis, as such did not communicate the necessary information to their clients. Participants who consume unpasteurized milk, unwashed vegetables, and undercooked meat had high prevalence of *T. gondii* antibodies compared to those who do not practice any of the aforementioned predisposing factors. Although cats are the definitive host of *T. gondii*, the result obtained in this study shows that by just owning a cat does not automatically makes you a victim of the parasite but the kind of hygiene practiced, food one eats and the processes involved in the food preparation.

Conclusion

Toxoplasma gondii is present among neonates born in Ahmadu Bello University Teaching Hospital, Shika, Zaria, Nigeria and can only arise as a result of congenital transmission from the mothers of theses neonates.

Recommendations

(i). Serological test for detecting *T. gondii* specific antibodies should be made compulsory to all pregnant women going for antenatal in hospitals to promote early detection of the parasites and adopting adequate precautionary measures to eradicate or limit their effects.
(ii). Government and non-governmental organization should help provide equipments and reagents that are needed in hospitals for performing test used in detecting *T. gondii* specific antibodies.
(iii). Serological test that can determine precisely how long *T. gondii* parasite has been present in one body should be developed to help assess the extent of damage and potential health defects that could arise.
(iv). Food and meats should be properly cooked before consumption especially pregnant women.
(v). Healthcare providers should educate pregnant women at their first prenatal visit about food hygiene and prevention of exposure to cat feces. Health education for women of child bearing age should include information about meat-related and soil-borne toxoplasmosis prevention.

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