

Original Research Article

Seroprevalence and risk factors associated with anti-*Toxoplasma gondii* antibodies in pregnant women attending antenatal clinics in Benue state, Nigeria

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ABSTRACT

Background: Toxoplasmosis causes serious economic losses in livestock. Congenital transmission can occur in pregnant women, commonly with serious consequences.

Methods: A cross-sectional study was designed to screen for the absence or presence of anti-*T. gondii* IgM or IgG antibodies and explore associated risk factors in pregnant women (n=261) that attended antenatal clinics in selected hospitals in Benue state, Nigeria. Blood samples were subjected to enzyme linked immunosorbent assay (ELISA). Information on sociodemographic data and possible risk factors were obtained using a structured questionnaire.

Results: Out of 261 pregnant women, 19 (7.3%) and 108 (41.1%) were seropositive for anti-*T. gondii* IgM and IgG respectively. Comparison of IgM and IgG results suggested that 4.4% presented evidence of possible primary infection, 38.7% previous exposure, 2.8% possible reactivated or chronic infections and 51.8% were susceptible to *T. gondii* infection. The nature of women's housing was significantly associated with anti-*T. gondii* IgM seropositivity ($p < 0.05$). Pregnant women that lived in mud houses with thatched roofs were three times more likely to be seropositive than those who lived in concrete/brick houses with aluminium/zinc roofs (OR=3.123, 95% CI:1.027, 9.495). The study also showed that 17.7% of apparent infections were attributable to the nature of housing. More than half of the women sampled were at risk of contracting primary toxoplasmosis.

Conclusion: Screening for anti- *T. gondii* antibodies during antenatal care is advocated in order to detect affected women so that appropriate management strategies can be implemented.

Keywords: Benue state, Enzyme linked immunosorbent assay, Seroprevalence, *Toxoplasma*

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by an obligate intracellular protozoan parasite, *Toxoplasma gondii*, from the phylum Apicomplexa.¹ *Toxoplasma gondii* infection is common and has a global distribution.¹ Members of the Felidae, especially cats who are the

definitive host of the parasite, primarily become infected by eating intermediate hosts harbouring tissue cysts. Intermediate hosts can include humans, birds and other warm-blooded animals.² Humans most commonly acquire infection by eating raw or undercooked meat containing tissue cysts, consuming water or food contaminated with cat faeces, or through accidental

ingestion of environmental oocysts.¹ Blood transfusion, transplacental transmission and organ transplantation are other sources of infection for humans.² Most infections in humans are asymptomatic, although pregnant women and immunocompromised individuals are at high risk from toxoplasmosis.³ Major sequelae in congenital infection include mental retardation, seizures, microcephalus, retinal necrosis, strabismus and retinochoroiditis in the newborn.³ Congenital toxoplasmosis may cause abortion, neonatal death, or foetal abnormalities with detrimental consequences for the foetus.^{4,5} It may also significantly reduce the quality of life in children who survive a prenatal infection.^{6,7}

Food habits, hand hygiene and management systems of animal production play important roles in the epidemiology of *T. gondii* infection. Epidemiological studies have shown that the prevalence of *T. gondii* infection in pregnant women varies greatly between countries. In Europe, prevalence can vary from 9.0 to 67.0%.⁸⁻¹³ In other countries such as Ghana levels as high as 92.0% have been reported, with high seroprevalence also described from human populations in Brazil and Venezuela.¹⁴⁻¹⁶ Far lower seroprevalence has been reported in Korea and Japan.^{17,18}

A combination of prenatal screening for anti-*T. gondii* antibodies, ultrasound and amniotic fluid polymerase chain reaction (PCR), as well as neonatal screening for antibody titres, has led to a correct diagnosis of congenital toxoplasmosis in 98% of cases.¹⁹ Serologic testing is usually performed first, detecting anti-*T. gondii* IgM and IgG antibodies. However, results of IgG and IgM testing can often be difficult to interpret. Serologic testing gives an indirect indication of infection status; whether recent or chronic. IgM antibodies are produced during the first week following infection and reach a plateau within 1 month.³

Levels of specific IgM antibodies usually decrease after 1 to 6 months but commonly remain measurable for a year or longer. In some cases, IgM can disappear within 3 months or be minimally detectable.³ Differences both in the individual immune response and in the characteristics of the technique used affect the kinetics of IgG detection.³ Whatever the method, IgG synthesis reaches a plateau within 2 or 3 months and then declines more or less rapidly and persists lifelong at residual titres, which are highly variable among patients.³

In Nigeria, human seroprevalence of 27 to 40.0 % has been reported in the last 10 years.²⁰⁻²⁴ An earlier study in Benue state reported a seroprevalence of 43.7 % in women of childbearing age.²⁵ More than twenty years later, information on the current prevalence is lacking. The present study was designed to screen for the absence or presence of anti-*T. gondii* IgM or IgG antibodies and establish associated risk factors in pregnant women in Benue state, Nigeria.

METHODS

Study area

This study was conducted in three selected state general hospitals and the Federal Medical Center (FMC) in Benue state. Benue state is in the north central region of Nigeria. It shares boundaries with five other states, namely; Nasarawa state to the north, Taraba state to the east, Cross-River state to the south, Enugu state to the south-west and Kogi state to the west, as well as the Republic of Cameroon to the south-east. The state experiences two seasons; a wet season from April to October, and a dry season from November to March. Benue State is divided into three senatorial/agricultural zones, namely; Benue north-east (zone A), Benue north-west (zone B) and Benue south (zone C). Inhabitants of the state commonly engage in farming, fishing and animal husbandry.²⁶

Sample size calculation

A sample size of 378 was calculated for this study using an expected prevalence of 43.7 %, based upon a previous publication.²⁵ However, only 261 women consented to join the study.

Sampling

A multi-stage sampling technique was used for the selection of pregnant women from the three zones of Benue state. Within each zone, samples were collected from the state general hospital within a selected local government area (LGA) and the FMC. Selected local governments included Makurdi, Kastina Ala and Otukpo. Hospitals were approached for their consent for inclusion in the study.

Inclusion criteria

Pregnant women attending antenatal clinic at the State general hospital in selected LGA and the FMC were eligible for inclusion in this study. Only those women who gave their consent were included.

Exclusion criteria

Non-pregnant women and pregnant women who did not give their consent were not included in the study.

Sample collection

Samples were collected between June and December 2016. Three millilitres of blood were collected aseptically from each participant via the median cubital vein by a trained medical laboratory scientist into an appropriately labelled plain vacutainer. Samples were kept in a cool box containing ice and transported to the Ahmadu Ali Center for Public Health and Comparative Medicine laboratory of the University of Agriculture Makurdi for

processing into sera. Sera from these blood samples were separated by allowing the blood to clot at room temperature prior to centrifugation at 3500 g for 10 minutes and storage at -20°C.

Serology

Sera samples were subjected to enzyme linked immunosorbent assay (ELISA) using a readily available commercial kit (abcam®, United Kingdom) to detect specific anti-*Toxoplasma gondii* IgM and IgG antibodies. The assay was validated and carried out according to the manufacturer’s instructions. Serology was carried out in the Veterinary Public Health and Preventive Medicine laboratory of Ahmadu Bello University, Zaria, Nigeria.

Assessment of putative risk factors

Structured questionnaires were used to collect sociodemographic data including the participant’s age, level of literacy, occupation and obstetrical history. Additional information included the participant’s major source of drinking water, contact with cats and soil, type of housing, type of meat consumed and whether meat was sampled while cooking. A pilot study was conducted to assess clarity of the questions. Instruments for the questionnaire survey included oral interview and participants who were literate filled and returned their forms.

Prior to commencement of the study ethical approval was obtained from the appropriate authorities. Informed consent was obtained from all study participants following explanation of the significance of the study. All participants were assured of confidentiality and anonymity and were requested to sign a document giving their permission to be included in the study.

Statistical analysis

Data obtained from the questionnaire and results of the laboratory analyses were entered into Microsoft® Excel and analysed using IBM SPSS® Statistics v.24, (New York, USA). Univariate binary logistic regression was performed to determine significant risk factors that associated with anti- *T. gondii* antibodies. The population attributable fraction (PAR) was computed as being calculated in a cross-sectional study. Statistical significance was set at a probability of 5% (p <0.05) with a confidence interval of 95%.

RESULTS

Sociodemographic characteristics

Out of 261 participants recruited for the study, sociodemographic information was obtained from 258 women. About 55% of the respondents were residing in Zone B. Result indicated that 91.5% of the respondents were between 20 to 39 years, 96.5% were married and

almost half of the respondents had secondary school education. Most of the respondents were housewives or self-employed. Reproductive history as reported by the participants of the study showed that more than half were in the third trimester of pregnancy, with the least (10.7%) in the first trimester. On average, 72.5 and 88.4% had no history of abortion or stillbirth, respectively (Table 1).

Table 1: Sociodemographic characteristics of pregnant women sampled in Benue state, Nigeria, 2016 (n = 258).

Variables	Category	N	Percentage(%)
Zone	A	58	22.5
	B	142	55.0
	C	58	22.5
Age	<20	16	6.2
	20-39	236	91.5
	≥40	6	2.3
Marital status	Single	9	3.5
	Married	249	96.5
Education	No formal education	17	6.6
	Primary	24	9.3
	Secondary	121	46.9
	Tertiary	96	37.2
Occupation	Student	30	11.6
	Housewife	71	27.5
	Trader	32	12.4
	Farmer	34	13.2
	Civil servant	16	6.2
	Private sector	15	5.8
	Self employed	60	23.3
	*Stage of pregnancy (n = 243)	First trimester	26
	Second trimester	84	34.6
	Third trimester	133	54.7
History of abortion	Yes	71	27.5
	No	187	72.5
History of stillbirth	Yes	30	11.6
	No	228	88.4

*= Total number of respondents who gave information on their stage of pregnancy.

Seroprevalence of anti-*T. gondii* antibodies

From 261 human sera samples, 7.3 and 41.1% were positive/reactive to anti-*T. gondii* IgM and IgG antibodies, respectively (Table 2). Further classification based on the combined humoral immune responses

indicated that 4.4% presented evidence of possible primary infection, 38.7% previous exposure, 2.8% possible reactivated or chronic infection and 51.8% were susceptible to *T. gondii* infection (Table 3).

Table 2: Detection of anti-Toxoplasma gondii antibodies among pregnant women studied in Benue State, Nigeria, 2016 (n = 261).

Antibody	Test result	Frequency	Specific rate (%)
IgM	positive	19	7.3
	negative	231	88.5
	inconclusive	11	4.2
IgG	reactive	108	41.4
	non-reactive	147	56.3
	inconclusive	6	2.3

Table 3: Summary of specific anti-Toxoplasma gondii antibody profiles among pregnant women sampled in Benue State, Nigeria, 2016.

Antibody profile	Tested		Inference
	No.	%	
IgM (+) IgG (-)	251	4.4	Possible primary infection
IgM (-) IgG (+)	248	38.7	Previous exposure
IgM (+) IgG (+)	254	2.8	Possible reactivated or chronic infection
IgM (-) IgG (-)	245	51.8	Susceptible

Population characteristics associated with T. gondii seroprevalence

Comparison of geographic region indicated no significant difference associated with zone (Table 4).

Table 4: Occurrence of anti-Toxoplasma gondii antibodies in relation to population characteristics assessed among pregnant women sampled in Benue state, Nigeria, 2016.

Variable	IgM			p value	IgG			p value
	NE	NP	%		NE	NP	%	
Senatorial zone				0.337 NS				0.975 NS
A	56	5	8.9		58	24	41.4	
B	138	8	5.8		137	59	43.1	
C	53	5	9.4		57	24	42.1	
Age group (years)				0.423 NS				0.837 NS
<20	15	2	13.3		16	6	37.5	
20-39	225	16	7.1		229	97	42.4	
≥40	6	0	0		6	3	50.0	
Education				0.656 NS				0.659 NS
No formal education	16	2	12.5		17	6	35.3	
Primary	23	2	8.7		24	10	41.7	
Secondary	115	10	8.7		118	55	46.6	
Tertiary	92	4	4.3		92	36	39.1	
Stage of pregnancy				0.926 NS				0.096 NS
1 st trimester	25	1	4.0		26	6	23.1	
2 nd trimester	80	6	7.5		81	38	46.9	
3 rd trimester	129	8	6.2		130	56	43.1	
History of abortion				0.268 NS				0.897 NS
Yes	68	7	10.3		67	28	41.8	
No	179	11	6.1		185	79	42.7	
History of stillbirth				0.980 NS				0.784 NS
Yes	27	2	7.4		29	13	44.8	
No	220	16	7.3		223	94	42.2	

%=percentage positive, NE=number examined, NP=number positive, NS=not significant. NE differs for IgM and IgG because of the difference in number of inconclusive results

The effect of age was also not statistically significant, although it was noted that IgM seropositivity decreased with age while IgG increased, likely reflecting the increased likelihood of historic infection with greater age.

Women with higher educational status presented with lower average IgM seropositivity, although the difference was also not statistically significant.

Table 5: Association between the nature of respondents housing and the presence of anti-Toxoplasma gondii IgM among pregnant women sampled in Benue State, Nigeria, 2016.

Nature of housing	Presence of anti-Toxoplasma gondii IgM		Total (N)	p value	OR	95 % CI of OR
	Positive (%)	Negative (%)				
Mud house with thatched roof	5 (16.7)	25 (83.3)	30	0.045	3.123	1.027-9.495
House built with concrete/bricks and aluminium or zinc roof	13 (6.0)	203 (94.0)	216			
Total	18 (7.3)	228 (92.7)	246*			

* Total number of subjects after exclusion of 4 samples which had no corresponding demographic data and 11 inconclusive results.

Table 6: Occurrence of anti-Toxoplasma gondii antibodies in relation to putative risk factors assessed among pregnant women sampled in Benue state, Nigeria, 2016.

Variable	IgM			IgG				
	NE	NP	%	p value	NE	NP	%	p value
Engagement in farm activities				0.977 NS				0.722 NS
Yes	99	6	6.1		99	44	44.4	
No	148	12	8.1		153	63	41.2	
Keep cat(s) at home				0.209 NS				0.066 NS
Yes	21	3	14.3		21	13	61.9	
No	226	15	6.6		231	94	40.7	
Encounter cats within home surroundings				0.569 NS				0.138 NS
Yes	98	6	6.1		103	38	36.9	
No	149	12	8.1		149	69	46.3	
Meat consumed*				NS				NS
Chicken	229	18	7.9	0.993	232	100	43.1	0.642
Pork	107	8	7.5	0.930	110	48	43.6	0.993
Beef	217	17	7.8	0.980	221	96	43.4	0.363
Mutton/chevon	184	15	8.2	0.980	188	80	43.0	0.579
Bush meat	86	6	7.0	1.000	86	39	45.3	0.950
Rat meat	24	1	4.2	0.848	24	11	45.8	0.978
Suya (smoked meat)	124	9	7.3	0.883	124	58	46.8	0.279
Sampling of meat while cooking				0.495 NS				0.895 NS
Yes	156	8	5.1		160	66	41.3	
No	90	10	11.1		90	41	45.6	
Primary source of drinking water				0.592 NS				0.739 NS
Well	55	3	5.5		51	21	41.2	
Stream/lake/river	4	0	0		5	2	40	
Tap water	40	0	0		43	23	53.5	
Borehole	48	2	4.2		43	16	37.2	
Sachet water	100	11	11.0		100	41	41.0	
Washing of raw vegetables/fruits before consumption				0.736 NS				0.448 NS
Always	218	16	7.3		221	96	43.4	
Most of the time but not always	20	1	5.0		22	7	31.8	
Most of the time I don't	4	0	0		4	3	75.0	
No	3	1	33.3		3	1	33.3	

%=percentage positive, NE=number examined, NP=number positive, NS=not significant, *=meat consumed 6 months before commencement of sampling. NE differs for IgM and IgG because of the difference in number of inconclusive results.

Risk factors associated with *T. gondii* seroprevalence

The nature of respondents housing was significantly associated with the presence of anti-*T. gondii* IgM antibodies ($p < 0.05$; Table 5). Pregnant women who lived in mud houses with thatched roofs were ~three-fold more likely to be seropositive (16.7%) compared to those who lived in concrete/brick houses with aluminium/zinc roofs (6.0%; OR=3.123, 95% CI: 1.027-9.495).

Comparison of the other risk factors assessed in this study did not reveal any significant associations with the presence of anti- *T. gondii* antibodies. These included primary source of drinking water, engagement in farming activities, whether they kept cat(s) at home, type of meat consumed, whether they sample meat while cooking and have contact with raw vegetables and fruits (Table 6).

Population attributable fraction

In this study, 17.7% of *T. gondii* infections were attributed to the nature of housing of the studied women.

DISCUSSION

Toxoplasmosis has long been recognised as a serious threat to public health. More than 20 years ago a serological study in women of childbearing age in Benue state, Nigeria, revealed a prevalence of 43.7% chronic *T. gondii* infection.²⁵ In the study described here we show that chronic *T. gondii* infection persists within the human population at a comparable level, suggesting that current public health practices have not reduced parasite transmission. The high seroprevalence detected is indicative of reoccurring exposure of women to circulating *T. gondii* bradyzoites in the food chain or oocysts in the surrounding environment. A contrasting result was observed in HIV/AIDS patients in the same state.²⁷

Comparison of IgM seropositivity revealed that a smaller proportion (7.3%) of the women appeared to be host to possible active infections. This may be explained by the fact that these women were at a high risk of infection due to the pregnancy-associated reduction in immunocompetence.

IgM antibodies are produced during the first week following infection and reach a plateau within one month, usually decreasing after one to six months.³ However, IgM antibodies have been shown to persist in some patients for months or years using immunosorbent agglutination assay (ISAGA), ELISA and indirect fluorescence antibody test (IFAT).²⁸ Thus, in the absence of a confirmatory IgG avidity test, the authors have used the word “possible” to indicate the likelihood of occurrence of a recent or reactivated infection.

Importantly, more than half of the women sampled were identified as susceptible to *Toxoplasma* infection. In the absence of an effective vaccine or routine chemoprophylaxis against toxoplasmosis in humans, this group of women are at risk of infection. The persistent level of chronic infection within the sampled population suggest limited knowledge or practise of preventive measures. This result is comparable to a study in Maiduguri, Borno state, which reported 52.7% of the sampled pregnant women to be susceptible to *T. gondii* infection.²³

The results from this study are comparable to studies in Lagos and Maiduguri that reported 7.6 and 8.9% for anti-*T. gondii* IgM, and 32.6 and 40.0% for IgG antibodies, respectively.^{21,23} However, our study results are unlike those conducted in Zaria, Sokoto and Osogbo which reported seropositivity of anti-*T. gondii* IgG as 29.1, 27.7 and 30.4%, respectively.^{20,22,24} In comparison with other studies conducted outside Nigeria, the present study reported a higher seroprevalence than those of Iran at 29.0%, Southern Ethiopia 23.9% and Egypt 20.4%.²⁹⁻³¹ However, other studies have reported higher seroprevalence in Brazil 77.5%, Columbia 63.5%, Algeria 47.8%, and Tanzania 44.5%.³²⁻³⁵ Variations in seroprevalence among different countries could be explained by differences in geographical and climatic conditions, food habits, personal hygiene and management systems of food animals, all of which play important roles in the epidemiology of *T. gondii* infection.

The current study reported a significant relationship between the occurrence of anti-*T. gondii* IgM and the nature of human housing. It indicated that pregnant women who live in mud houses with thatched roofs were at a significantly greater risk of acquiring *T. gondii* infection if they have not been exposed before, than those who lived in houses built with concrete/bricks with aluminium or zinc roofs. It is possible that the flooring in mud houses or thatched roofs encourage the easy spread or persistence of *Toxoplasma* oocysts, thus increasing the risk of *T. gondii* infection, particularly with concurrently poor hand hygiene. The nature of housing may only be a predictor of spread and not necessarily a risk factor.

The present study reports increased anti-*T. gondii* IgG seropositivity with increasing age, mirrored by decreased IgM seropositivity with age. However, there was no significant association between presence of anti- *T. gondii* antibodies and human age. This finding can likely be explained by the extent of exposure of older women to circulating bradyzoites or *T. gondii* oocysts in the environment. Increased age indicates a greater likelihood of previous exposure to infection, developing antibody responses which can persist for many years. IgG antibodies have been shown to reach peak production two

or three months after infection and then decrease rapidly before persisting lifelong at residual titers.³ Thus, new infections are more likely to be observed in younger women. These findings are consistent with many other studies.^{20,37-39,24} However, there has been a report of significantly increased anti- *T. gondii* IgM seropositivity with age in pregnant women.²⁴ The relationship between age and *T. gondii* infection as observed in the current study disagrees with some other reports.^{23,40} The current study suggested a decrease in anti- *T. gondii* IgM seropositivity and an increase in IgG seropositivity as the educational status of the sampled women increased, although the association was not statistically significant. This trend is consistent with some other reports of IgM and IgG seropositivity and IgM seropositivity only.^{23,24} The decrease in IgM seropositivity in educationally advanced women could be related to the well-informed status of these women on ways to prevent infection and on basic hygiene, while the previous exposure to circulating bradyzoites or oocysts of *T. gondii* in the environment accounts for the increase in chronic infections. It is possible that increased educational status is reflected by greater geographic movement, increasing previous opportunities for chronic infection.

Findings from the present study indicated notable anti- *T. gondii* IgM and IgG seropositivity throughout gestation. An implication of this result is that there is a considerable danger of congenital transmission for women with primary and reactivated infections acquired during pregnancy, with resultant grave consequences for the foetus including abortion, brain and visual disorders.³ The placental barrier is known to be efficient at preventing disease transmission from mother to child in early pregnancy.³ As pregnancy progresses, the placenta becomes more permeable allowing parasite transmission in about 30% of cases in the second trimester and 60-70% of cases in third trimester and even near the period of delivery.³ These findings differ from other published reports.^{21,23}

CONCLUSION

The present study has reported a high seroprevalence of apparently chronic *T. gondii* infection in pregnant women of Benue state, Nigeria, indicative of consistently high levels of exposure within the human population. A small proportion of women may have become infected a few months before sampling. Due to the serious consequences that arise from congenital toxoplasmosis, it is pertinent that health authorities in Benue state consider laboratory testing for anti-*T. gondii* antibodies during antenatal care and to educate women about the disease and ways to reduce the risk of infection.

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Conflict of interest: None declared

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