

Sero-Prevalence and Factors Associated with Toxoplasma Gondii Infection among Pregnant Women Attending Antenatal Care in the Referral Hospital in Tanzania: Cross Sectional Study

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Abstract

Background: Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*. It is estimated that more than one third of the global population is infected with this parasitic infection. Serological screening of pregnant women for the presence of *T. gondii* antibodies is not part of the antenatal care package in Tanzania. The current study assessed sero-prevalence and associated factors for *T. gondii* infection among pregnant women attending antenatal care clinic in KCMC referral Hospital.

Methods: Between April 26th to May 18th 2014 a total of 144 pregnant women attending KCMC for antenatal care were enrolled and screened for IgG and IgM antibodies against parasite *T. gondii* using lateral flow chromatography Assay which detects and differentiates IgM and IgG anti-*T. gondii* in human serum or plasma. Positive IgG results meaning, at some point in the life someone has been exposed to *T. gondii* and doesn't mean the active infection.

Results: Of 144 expectant women investigated, 60 (41.7%) were seropositive for *T. gondii*-specific antibodies. Among sero-positive pregnant women 1 (1.7%) had anti-Toxoplasma IgM indicating acute infection. The risks of contracting *T. gondii* was less by nearly 50% among pregnant women 30-years-old and above compared with those below 30-years-old (Crude OR=0.45; 95% CI 0.23-0.88; p=0.2). There was no statistical evidence for the difference (p=0.059) among *T. gondii* positive women across different age groups. HIV infection was not statistically significant associated with *T. gondii* infection [adjusted OR 1.17; CI 0.42-3.24; p=0.764.

Conclusions: One third of pregnant women attended at antenatal clinic were seropositive for *T. gondii* at some point in their life which might increase neonatal complications. Due to complications related to Toxoplasmosis during pregnancy in neonatal period: such miscarriage, chorioretinitis, hydrocephalus, cerebral calcification and fetal death. There is urgent need for screening during antenatal care.

Keywords: Toxoplasmosis; Trimester; Congenital; Hydrocephalus; Northern Tanzania

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Background

Toxoplasmosis is prevalent worldwide where by more than 70% of global population is infected with the parasite [1-4]. Human infection can result from ingesting undercooked meat containing

Toxoplasma cysts. Another mode of transmission is from direct contact with cats' litter or consuming food contaminated by oocysts excreted in the feces of infected cats. Its spread is influenced by various factors such as climate conditions, immune status and cultural behaviours of individual's hygiene, type of food, cooking

methods among others [4-6]. Presence of this infection during pregnancy, especially during the third trimester can lead to serious neonatal complications. The most pronounced complications are miscarriage, chorioretinitis, hydrocephalus, cerebral calcification and fetal death [7,8]. There is increased risk of congenital transmission among pregnant women in the third trimester as compared to first trimester [9,10]. There is uneven distribution of *T. gondii* prevalence among pregnancy and childbearing age from different parts of the world. In Europe, the prevalence of Toxoplasmosis varies between 20%-50% in the South and between 50%-70% in the West, whereas in the humid region of Africa, the prevalence varies [11]. The poor socioeconomic status in many countries in Latin America, Asia and Africa has been the primary risk factor as it has been association with poor hygiene and unreliable safe water use [12]. Presence of cat in the house and poor hygiene during handling of cats' litter exposes these individuals to Toxoplasmosis infection especially for pregnant women who are the group of interest [10,12,13].

In Tanzania, only two studies have been conducted on the prevalence of Toxoplasmosis, in Dar es Salaam [14,15] the prevalence of Toxoplasmosis among pregnant women was 35% while Mwanza showed the prevalence of 30.9% respectively which seems to concur with other part of the world. This means more fetus are at increased risks to develop complications from Toxoplasmosis [16,17]. To prevent maternal-fetal transmission of Toxoplasmosis can be achieved through screening at the antenatal clinics and treat by using drugs such as spiramycine to prevents its complications [17]. Since there is no information on the prevalence of Toxoplasmosis among pregnant women in Kilimanjaro, this study was conducted in order to determine seroprevalence of *T. gondii* and associated factors to justify the need for screening among pregnant women in Tanzania.

Material and Methods

Study design and population

A cross-sectional hospital based study was conducted from April 26th to May 18th 2014 in Kilimanjaro Christian Medical Centre (KCMC) in North-eastern Tanzania. The hospital's where this study was conducted; deliver services to about 15 million people in Northern part of Tanzania. In this region most of employed/business people consume pork and beef, this might be undercooked especially when grilled at bars. Permission to conduct this study was granted by Kilimanjaro Christian Medical University College ethical review committee. All women signed an informed consent prior to enrolment. Pregnant women attending antenatal care clinic were enrolled consecutively into the study during the study period. Inclusions criteria were all pregnant women who were residing in Moshi during study period while exclusion criteria were those who were residing in Moshi for less than three months. A full medical history including socio-demographic characteristics, presences of chronic illness and eating habit was recorded.

Samples

The samples were collected by aseptic procedures from each among the 144 pregnant women. About 4mls of venous blood

were drawn in plain tubes, and transported from antenatal clinic to clinical laboratory. All samples were left at to coagulate for 30 minutes, and then separated by centrifugation at 3000 rpm for 4minutes to obtain sera. Sera were stored at -20 °C till analysis.

ELISA reagents and procedure

All samples were tested for detection of IgG antibodies to *T. gondii* using ELISA commercial kit (Human Gesellschaft fur Biochemica und Diagnostica mbH, Max-Planck-Ring, Wiesbaden-German). Serum samples was diluted 1:100 (5 μ L: 450 μ L) with dilution buffer, 100 μ L of diluted samples were then applied to a coated microtiter plate, and incubated for 30 minutes at room temperature. The plate was then washed 4 times, with 350 μ of 1:20 dilution washing buffer using automated ELISA washer (Lx50 BioTek, Highland park, Winooski, USA). Then, 100 μ L of conjugate was added, and incubated for 30 minutes, at room temperature, then washed 5 times as described above. 100 μ L of substrate solution was added, and incubated in dark for 15 minutes, and then the reaction was stopped by 100 μ L of stop solution. Plate was read at 450 nm soon after adding stop solution using ELISA reader (Lx800 BioTek, Highland Park, and Winooski, USA). Results were interpreted as positive when the sample OD \geq MCC + 15%, and negative when sample OD < MCC - 15%, as per manufacturer instructions.

Rapid test

All positive samples from IgG ELISA assay were re-tested by using a rapid test kit (Toxo IgM/IgG lateral flow chromatography immunoassay, Houston, TX USA), a lateral flow chromatography assay which detect and differentiate between IgM and IgG antibodies to *T. gondii* in human serum, according to manufacturer's instructions.

Data analysis

All statistical analyses were performed using a commercially available statistical package STATA version 12.1(StataCorp LP, College Station, TX). Categorical variables were summarized using cross tabulation to estimate different proportion. The effect of social-demographic characteristics and eating habit of meat on primary outcome of the study (Toxoplasmosis infection) was assessed using bivariate analysis. Explanatory variables were included in the multivariate analysis if the variable had p-value < 0.2 in bivariate analysis. Logistic regression model were used to estimate the odds ratio (OR) and 95% confidence interval (CI) to evaluate the strength of association between explanatory variable and primary outcome. Two sided Wald test p-value are presented.

Results

A total of 144 pregnant women were enrolled during the study period majority of them (55%) were aged 30yrs and above. About fifty six percent of the studied participants were from urban residence 81/144, only 36% were employed the rest were doing business and agriculture activities. On gravidity, about 87/144 (60.42%) were multigravid while 65/144(45.14%) were at their third trimester. Most of the study participants 82/144 (60%) had secondary education and above (**Table 1**). Of the 144 pregnant

women studied, 60 (41.67%) were found seropositive for anti-Toxoplasma IgG antibodies, indicating chronic infection (Table 1). Out of seropositive study participants, one woman (0.69%) had anti-Toxoplasma IgM, indicating acute infection for Toxoplasmosis (Table 1).

The risks of contracting *T. gondii* was less by nearly 50% among pregnant women 30years and above compared with those below 30yrs (Crude OR=0.45: 95% CI 0.23-088; p=0.2). There was no statistical evidence for the difference (p=0.059) among *T. gondii* positive women across first and third trimester among studied pregnant women because the exactly time of infection was not defined (Figure 1). HIV infection was not statistically significant associated with *T. gondii* infection (adjusted OR 1.17: CI 0.42-3.24; p=0.764 (Table 2). On multivariate logistic regression analysis there was significant difference in Toxoplasma seropositivity among pregnant women who are younger as compared to elderly ones (53.31% versus 32.91%) (OR=0.32, 95% CI: 0.23-0.88, p=0.018). Potential factor HIV seropositivity was subjected into multivariate logistic regression analysis but there was no association. Occupation of the pregnant women was subjected

to logistic regression analysis but there was no association even when was associated with residence, as majority of employed and business women were living in urban areas as compared to peasant who were residing in rural settings. Contact with domestic cat was exposed to both Univariate and multivariate logistic regression but there was no significant association with Toxoplasma infection among pregnant women.

Discussion

This was among few studies in Tanzania, and the first study to be conducted among pregnant women in North-eastern part of the country to determine the actual scenario of Toxoplasmosis among this population. The seroprevalence of *T. gondii* infection in this study was found to be higher than lake zone (30.9%) and (35%) reported in Mwanza and Dar es Salaam respectively. But the prevalence was lower compare to 46% among abattoir workers, livestock keepers and animal health workers in Tanga. The different in prevalence might be explained by the fact that, these people were exposed to animal and soil environment hence more chance to contact infection as compared to pregnant women from different professionals [15]. The different could partly be explained by climatic conditions where Moshi has more rainy seasons compared with Dar es Salaam and Mwanza Tanzania. This conditions favours sporulation of oocysts; the methodology which was used in the current was ELISA which is more specific as compared to immunosorbent agglutination which was used in Dar es Salaam [16,17]. Age different among study participants was also associated with Toxoplasmosis infection; where by younger women were more infected compared to elderly ones. This finding was different from other studies which showed that there was an increased risk of infection as age increases [18]. This might be explained by the facts that younger people prefer outing compared to elderly, this outing expose them to grilled meat (which might be undercooked), fruits and saladies which may be contaminated with the parasites hence increased risks of infection [19-21]. In the current study we could not extrapolate

Table 1 Distribution of *T. gondii* sero-prevalence with demographic characteristics among pregnant women (N=144), 2014 in Moshi.

Patient's Characteristic	ELISA-IgG Test for <i>T. gondii</i>			
	Negative		Positive	
Age group(years)	No	Row%	No	Row%
18-(n=65)	31	47.7	34	52.3
30-(n=79)	53	67.1	26	32.9
Residence				
Rural (n=63)	37	58.7	26	41.3
Urban (n=81)	47	58.0	34	42.0
Education				
Primary level (n=62)	30	48.4	32	51.6
Secondary and above (n=82)	54	65.9	28	34.1
Occupation				
Business (n=32)	18	56.2	14	43.8
Employed (n=52)	36	69.2	16	30.8
Peasant (n=60)	30	50.0	30	50.0
Gravidity				
Primigravid (n=46)	25	54.3	21	45.7
Multigravid (n=87)	52	59.8	35	40.2
Grandmultigravid(n=11)	7	63.6	4	36.4
HIV status				
Negative (n=125)	73	58.4	52	41.6
Positive (n=19)	11	57.9	8	42.1
Undercooked meat				
No (n=99)	57	57.6	42	42.4
Yes(n=45)	27	60.0	18	40.0
Trimester				
1st Trimester (n=22)	14	63.6	8	36.4
2nd Trimester(n=57)	39	68.4	18	31.6
3rd Trimester (n=65)	31	47.7	34	52.3
Contact with cat				
No (n=95)	55	57.9	40	42.1
Yes (n=49)	29	59.2	20	40.8

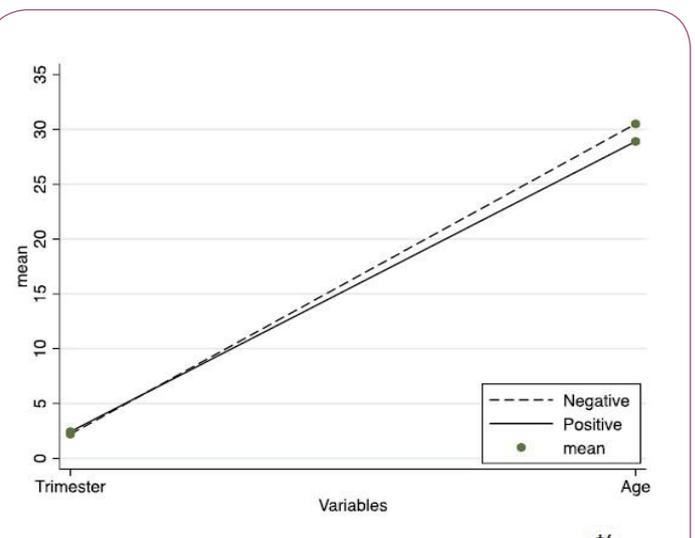


Figure 1 Sero-Prevalence of *T. gondii* by years and trimester among 144 women attending GOPD KCMC, 2014.

Table 2 Factors associated with *T. gondii* sero-prevalence among 144 pregnant women 2014 in Moshi.

Variable	Toxoplasmosis infection		Crude OR [95% CI]	p-value	Adjusted OR ^a [95% CI]	p-value
	No n (%)	Yes n (%)				
Age (years)						
Below 30	31 (36.9)	34 (56.7)				
≥ 30	53 (63.1)	26 (43.3)	0.45 [0.23–0.88]	0.020	0.41 [0.20–0.84]	0.014
Residence						
Rural	37 (44.0)	26 (43.3)				
Urban	47 (56.0)	34 (56.7)	1.03 [0.53–2.01]	0.932	1.18 [0.54–2.60]	0.675
Education						
Primary	30 (35.7)	32 (53.3)				
Secondary	54 (64.3)	28 (46.7)	0.49 [0.25–0.96]	0.036	0.64 [0.24–1.68]	0.362
Occupation						
Peasant	30 (35.7)	30 (50.0)				
Employed	36 (42.9)	16 (26.7)	0.44 [0.02–0.97]	0.004	0.61 [0.20–1.86]	0.389
Business	18 (21.4)	14 (23.3)	0.77 [0.33–1.84]	0.57	1.10 [0.42–2.89]	0.841
Gravidity						
Primigravid	25 (29.8)	21 (35.0)				
Multigravid	59 (70.2)	39 (65.0)	0.79 [0.39–1.60]	0.507	1.26 [0.51–3.09]	0.620
Trimester						
First	14 (16.7)	8 (13.3)				
Second	39 (46.4)	18 (30.0)	0.81 [0.29–2.27]	0.685	0.881 [0.31–2.74]	0.881
Third	31 (36.9)	34 (56.7)	1.92 [0.71–5.19]	0.199	2.52 [0.85–7.51]	0.097
HIV						
Negative	73 (86.9)	52 (86.7)				
Positive	11 (13.1)	8 (13.3)	1.02 [0.38–2.71]	0.967	1.17 [0.42–3.24]	0.764
Undercooked meat						
No	57 (67.9)	42 (70.0)				
Yes	27 (32.1)	18 (30.0)	0.90 [0.44–1.85]	0.784	0.79 [0.37–1.70]	0.553
Contact with Cat						
No	55 (65.5)	40 (66.7)				
Yes	29 (34.5)	20 (33.3)	0.95 [0.47–1.91]	0.882	1.04 [0.50–2.18]	0.919

exactly time of infection, across three trimester, which is one of the weakness of our study compared to other studies. [10,22]. Contact with cat litter is another contributing risk for *T. gondii* infection [23], in the current study about one third of the study participants were keeping house cat but there was no significant association between *T. gondii* infection and history of cat contact was found. This is because presence of cat in the house is not enough to confirm zoonosis, rather handling of cats' litter is of more important. These findings were consistence with studies which was conducted in Mwanza Tanzania [17], Palestine [24] and Zambia [25]. But other studies from Ethiopia [26] and Taiwan [27] showed a significant association between contact with cats and seroprevalence of *T. gondii*. The presence of one woman with active infection who wasn't aware with the disease means more risks to the fetus, this stress on the significant of screening to control congenital transmission and its outcome. Apart from consequences among pregnant women [28], in general population Toxoplasmosis is associated with risks to develop schizophrenia and reduce intelligence which compromise social adaption of infected person [4,29]. Immune compromised people are more prone to clinical Toxoplasma

[30], in the current study about thirteen percent of the studied women were HIV positive but there was no statistical significant association between HIV seropositivity and positive ELISA for Toxoplasmosis. This might be partially be explained by small sample size as well as most of the women were on ART hence reduced chance for the opportunistic infections .

Conclusions

This study confirmed seroprevalence of *T. gondii* among pregnant women in North-eastern Tanzania, which suggests the need for intervention, especially with regards to education on the associated factors for the purpose of reducing morbidity and mortality. Despite the lack of statistical correlation between infection and associated risk factors, preventive measures among pregnant women should be followed to prevent consequences of Toxoplasmosis.

Author's Contributions

ERS designed the project. ERS, EGK, GM and SNN collected specimens and clinical data. EGK and SNN analysed and interpreted the results. ERS, TS and DM analysed and interpreted

the data. ERS wrote the draft of the manuscript which was approved by all authors. All authors read and approval the final version of this manuscript.

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