

Seroprevalence and risk factors of *Toxoplasma gondii* infection among pregnant women attending antenatal care in Kigali, Rwanda

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Abstract

Background: *Toxoplasma gondii* infection in pregnancy, if left untreated, is associated with spontaneous abortions, low birth weight babies, congenital deformities and intrauterine deaths. The infection is also associated with human immune deficiency virus/acquired immunodeficiency syndrome (HIV/AIDS). In Rwanda, the burden and risk factors of *T. gondii* infection among pregnant women and among HIV infected pregnant women is largely unknown. This cross-sectional study aimed at determining the seroprevalence of *T. gondii* infections and their risk factors among pregnant women in Kigali, Rwanda.

Methods: Pregnant women aged 18 years and above who were attending antenatal care at four clinics in Kigali City, between April and August 2014 were screened for IgG and IgM antibodies against *T. gondii* using ELISA technique. Information on their HIV status and CD4⁺ cell count was obtained from their medical records. Participants were also interviewed on selected behaviours that predispose individuals to *T. gondii* infection.

Results: A total of 384 pregnant women were involved in the study. The overall *T. gondii* seroprevalence was 12.2%. Of the 384 pregnant women studied, 37 (9.6%) were positive for anti-*T. gondii*-specific IgG antibodies, indicating past infection and 15 (3.9%) had positive IgM results indicating recent infection. Drinking untreated water and eating undercooked meat were identified as important risk factors for *T. gondii* infection respectively at 22.4% and 22.3% [OR=3.95, CI:2.09-7.49; p<0.001 and OR=3.27, 95% CI: 1.75-6.09; p<0.001].

Conclusion: Although the seroprevalence of *T. gondii* antibodies is relatively low, institution of interventional measures is desirable.

Keywords: *Toxoplasma gondii*, seroprevalence, pregnancy, risk factors, Rwanda

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite that causes toxoplasmosis (Jones *et al.*, 2001). It is estimated that about one third of the world's population is infected with *T. gondii* (Pappas *et al.*, 2009). In Africa, overall seroprevalence rate as high as 92.5% has been reported (Ayi *et al.*, 2009). However, the prevalence of infection varies widely between countries (from 10% to 80%) and often within a given country or between different communities in the same region (Pappas *et al.*, 2009). The variations in socio-economic factors between communities and countries have been described to account for the observed difference in prevalence (Rosso *et al.*, 2008; Fernando *et al.*, 2008).

Majority of horizontal transmissions to humans is due to either the ingestion of tissue cysts in infected meat or by the ingestion of soil, water, or food contaminated with sporulated oocysts from the environment or, less frequently, directly from feline faeces (Foulon *et al.*, 1999). Trans-placental transmission occurs when an immunocompetent woman acquires a primary infection during pregnancy (Hegab & Al-Mutawa, 2003), or may also be due to a reactivated infection in immunocompromised women (Dubey & Jones, 2008). Congenital infection can lead to a wide variety of manifestations in the foetus and infant including spontaneous abortion, still-birth, hydrocephalus or microcephalus, cerebral calcifications and retinochoroiditis (Gibbs, 2002; Goldenberg & Thompson, 2003).

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Seroprevalence of *T. gondii* among pregnant women has been studied by different researchers. A study done in Mwanza, Tanzania, showed a seroprevalence of 30.9% for *T. gondii*-specific antibodies (Mwambe *et al.*, 2013), while in Nigeria expectant women were reported to have a seroprevalence of 7.6% and 32.6% for *Toxoplasma* IgM and *Toxoplasma* IgG respectively (Deji-Agboola *et al.*, 2011). Despite the fact that *T. gondii* infection in pregnancy is associated with significant morbidities, serological screening of pregnant women for this infection is however, not routinely carried out during antenatal care in Rwanda. As a result, little is known about the prevalence and risk factors of the parasite among pregnant women in the country. Thus, the objective of the present study was to determine the seroprevalence of *T. gondii* infections among pregnant women attending selected antenatal clinics in Rwanda and risk factors associated with the acquisition of the infection.

Materials and Methods

Study area and population

This cross-sectional study was conducted in Kigali, Rwanda in four selected public Health Centres namely Biryogo, Cor-Unum, Muhima and Nyarugunga between April to August 2014. The Health Centres were selected because they were located in the central part of Kigali city and therefore likely to attract different categories of the population. The study population was all the pregnant women aged 18 years and above who were attending antenatal care at these health centres during the study period. Excluded from the study were non-pregnant women, and pregnant women who were below the age of 18 years.

Sample size and sampling procedures

The sample size of 384 pregnant women was calculated by using the formula by Cochran (1963) with an estimated prevalence (p) of 50%. The eligible women were requested to enrol into the study voluntarily on the day of their antenatal visit. The enrolment continued until the sample size of 384 was reached. Demographic data consisting of age, residence, occupation, marital status, education, and relevant clinical information such as gravidity and trimester were collected using a standardized data collection tool.

Sample collection and laboratory processing

About 5ml of venous blood was collected aseptically from each of the 384 pregnant women and transported to the National Reference Laboratory (NRL) in Kigali. At the NRL plasma was prepared from the whole blood by centrifugation at 3,000 rpm for 5 minutes and assayed for anti-*T. gondii* antibodies (IgM and IgG) using bio ELISA TOXO IgG & IgM kits (Biokit, Spain, Lot Number B 23643), according to the manufacturer's instructions.

A volume of 10 μ l of test serum was diluted using 1ml of sample diluent and the diluted specimen incubated in microplate wells coated with rabbit antibodies anti-human IgM or IgG. The wells were then washed using washing solution to remove residual test sample, and 100 μ l of *Toxoplasma* antigen labelled with peroxidase was added. The plates were then washed again using washing solution to eliminate unbound material. Furthermore, 100 μ l of solution of enzyme substrate and chromogen were added. This solution developed a blue colour if the sample contained anti-*T. gondii* IgM or IgG. The blue colour changed to yellow after blocking the reaction with sulphuric acid. The plates were then read using an ELISA reader at 450 nm wavelength. The concentration of antibodies in the sample was then determined by means of a calibration curve.

The cut-off value of the assay was calculated and results were expressed in an index by dividing sample absorbance by the cut-off value. The test was considered negative if the index was <0.9, the result was equivocal when index was from 0.9 to <1.0 while a positive result was indicated if the index was \geq 1.0. A negative reaction was judged as indicative of the absence of significant *Toxoplasma* antibodies. A positive *Toxoplasma* IgG reaction was interpreted as an indication of

either a past or recent infection. Information on HIV status and CD4+ cell count of the participants was obtained from their medical records.

Data analysis

Data collected were checked for completeness and consistency and were entered into a computer and analysed using SPSS version 17.0 software package. Likely association between the socio-demographic characteristics of participants and sero-positivity was assessed using a Chi-square test. To determine which of the characteristics could be having independent influence on observed *T. gondii* sero-positivity, those that were found to have a p-value of <0.2 were taken into the univariable and multivariable models. The manual backward-stepwise elimination of factors was used to develop a final model until the remaining variables were significant at p-value <0.05.

Ethical considerations

Ethical clearance was obtained from Kenyatta National Hospital-University of Nairobi Ethics and Research Committee and Rwanda National Ethics Committee. Permission was obtained from relevant authorities in all respective study sites before beginning the study. Written informed consent was sought from pregnant woman prior to involvement in the study. Only those who agreed to participate by signing the informed consent form were included in the study. Information about each study participant was kept confidential and venous blood specimens were stripped of all personal identification.

Results

A total of 384 pregnant women were enrolled during the study period. The median age of the study population was 27 years (range= 18 to 45 years). Most women (51.0%) were aged between 26 and 35 years. Over half (57.8%) of the study subjects were single, 63.5% were unemployed, and 64.1% were multigravidae with 54% on second trimester of their pregnancy. Majority of women (71.1%) had primary education, and only (25.0%) had attained a higher level of education (Table 1).

Table 1: Social-demographic and clinical characteristics of pregnant women (N=384)

Variable	Response	Number	Percentage
Age	18-25	156	40.6
	26-35	193	50.3
	36-45	35	9.1
Marital status	Married	162	42.2
	Single	222	57.8
Occupation	Employed	140	36.5
	Not employed	244	63.5
Level of education	None	15	3.9
	Primary	273	71.1
	Secondary and above	96	25.0
Gravidity	Primigravida	138	35.9
	Multigravida	246	64.1
Trimester of pregnancy	First	80	20.8
	Second	207	54.0
	Third	97	25.2
HIV status	Negative	365	95.0
	Positive	19	5.0
Water type	Treated	250	65.1
	Untreated	134	34.9
Own a cat	No	345	89.8
	Yes	39	10.2
Under cooked meat	No	272	70.8
	Yes	112	29.2

Eat in restaurant	No	220	57.3
	Yes	164	42.7
Taste food	No	97	25.3
	Yes	278	74.7
Had experience abortion	No	311	81.0
	Yes	73	19.0
Knowledge on <i>Toxoplasma gondii</i>	No	375	97.7
	Yes	9	2.3

Overall, out of the 384 pregnant women studied, 47 were sero-positive giving a sero-prevalence of *T. gondii* of 12.2%. A total of 37 (9.6%) were positive for anti-*T. gondii*-specific IgG antibodies, indicating past infection and 15 (3.9%) had positive IgM results indicating recent infection. Of all the characteristics of pregnant women studied, significant association with sero-positivity was found only in water used for drinking ($\chi^2=19.73$; $p < 0.001$) and consuming undercooked meat ($\chi^2=14.96$, $p < 0.001$) (Table 2).

Table 2: Chi-squared and Fisher's exact tests of characteristics for association with sero-positivity of *T. gondii*

Variable	Response	No. Positive (%)	No. Negative (%)	χ^2	p-value
Age (years)	18-25	18(11.5)	138(88.5)	0.87	0.65
	26-35	23(11.9)	170(88.1)		
	36-45	6(17.1)	29(82.9)		
Marital Status	Married	19(11.7)	143(88.3)	0.07	0.79
	Not Married	28(12.6)	194(87.8)		
Occupation	Unemployed	30(12.3)	214(87.7)	0.0	0.96
	Employed	17(12.1)	123(87.9)		
Education	None	4(26.7)	11(73.3)	3.21	0.2
	Primary	33(21.1)	240(87.9)		
	≥Secondary	10(10.4)	86(89.6)		
Gravidity	Primigravida	17(12.3)	121(87.7)	0.0013	0.97
	Multigravida	30(12.2)	216(87.8)		
Trimester of pregnancy	First	8(10.0)	72(90.0)	0.5154	0.77
	Second	26(12.6)	181(87.4)		
	Third	13(13.4)	84(86.6)		
HIV status	Negative	46(12.6)	319(87.4)		0.49
	Positive	1(5.3)	18(94.7)		
Water treatment	Treated	17(6.8)	233(93.2)	19.73	< 0.001
	Untreated	30(22.4)	104(77.6)		
Cat ownership	No	44(12.8)	301(87.2)		0.45
	Yes	3(7.7)	36(92.3)		
Consumption of undercooked meat	No	22(8.1)	250(91.9)	14.96	< 0.001
	Yes	25(22.3)	87(77.7)		
Eat in restaurant	No	21(9.5)	199(90.5)	3.48	0.062
	Yes	26(15.9)	138(84.1)		
Taste cooking food	No	16(16.5)	81(83.5)	2.19	0.14
	Yes	31(10.8)	256(89.2)		
Experienced abortion	No	35(11.3)	276(88.7)	1.48	0.224
	Yes	12(16.4)	61(83.6)		
Knowledge of toxoplasmosis	No	0	9(100)		0.608
	Yes	47(12.5)	328(87.5)		

In univariable analysis higher risk of infection with *T. gondii* increased with drinking untreated water (OR 3.95, CI 2.09-7.49); and consumption of undercooked meat (OR 3.27, CI 1.75-6.09). Eating in restaurant, and tasting cooking remained statistically not significant at $p < 0.05$. Since they had a $p < 0.2$ they were put in a multivariable logistic model along with the significantly associated variables (Table 3). Still, significantly associated with *T. gondii* sero-positivity were the drinking of

untreated water (Adjusted OR 4.01, CI 2.08-7.69, $p < 0.001$) and consumption of undercooked meat (Adjusted OR 3.27, CI 1.75-6.09, $p < 0.001$).

Table 3: Logistic regression of variables significantly associated with *T. gondii* sero-prevalence (N=384)

Variable	Response	Positive (%)	Negative (%)	Univariable		Multivariable	
				COR(95% CI)	P-value	AOR(95% CI)	P-value
Water treatment	Treated	17(6.8)	233(93.2)	1		1	
	Untreated	30(22.4)	104(77.6)	3.95(2.09-7.49)	<0.001	4.01(2.08-7.69)	<0.001
Eating of undercooked meat	No	22(8.1)	250(91.9)	1		1	
	Yes	25(22.3)	87(77.7)	3.27(1.75-6.09)	<0.001	3.32(1.74-6.32)	<0.001
Eat in restaurant	No	21(9.5)	199(90.5)	1		-	
	Yes	26(15.9)	138(84.1)	1.79(0.97-3.3)	0.062	-	
Taste cooking food	No	16(16.5)	81(83.5)	1		-	
	Yes	31(10.8)	256(89.2)	0.61 (0.31-1.18)	0.14	-	

Key: COR= Crude Odds Ratio; AOR= Adjusted Odds Ratio; CI= Confidence Interval

Discussion

Few studies have reported on seroprevalence of anti-*T. gondii* in Rwanda and none on pregnant women. The present study found an overall sero-prevalence of 12.2% for anti-*T. gondii* antibody among pregnant women attending antenatal clinics in Kigali, Rwanda. This rate is lower than the range of 25% to 92.5% seroprevalence among pregnant women reported elsewhere in Africa (Mwambe *et al.*, 2013; Alsammani, 2016). Various factors have been attributed to disparity in locality seroprevalence rates of *T. gondii* including, among others temperature, humidity, wind, where cooler weather conditions as found in Kigali would relatively disfavour the thriving of the parasite (Tenter *et al.*, 2000). Such regional variation of seroprevalence of *T. gondii* infection has previously been described (Jones *et al.*, 2001; Mwambe *et al.*, 2013). In addition, the observed differences in seroprevalence of anti-*T. gondii* infection may be due to cultural variations in the populations. Likewise, socio-economic status, education, sanitary conditions and differences in dietary habits may contribute to observed differences in sero-positivity (Petersen *et al.* 2010).

Surprisingly, in the present study, there was a non-statistically significant increase in sero-positivity of anti-*T. gondii* antibody with age of study participants. However, the observation is in agreement with other similar studies in which increasing age was more associated with chronic infection (Rosso *et al.*, 2008, Al-Mohammad *et al.*, 2010; Markovich *et al.*, 2014). This could be explained by the fact that older women are more likely to have been exposed much longer to any one of the risk factors than younger women.

Corroborating many other studies, the use of untreated water was found to increase the risk of infection with *T. gondii* (Mwambe *et al.*, 2013; Alsammani, 2014) through the ingestion of oocysts in contaminated water. A higher prevalence of *T. gondii* infection has been found among pregnant women who drink water from the well than those who drink piped water (Ishaku *et al.*, 2009). A similar finding showing an association between prevalence of *T. gondii* infection and source of drinking water has been reported from Brazil (Bahia-Oliveira *et al.*, 2003).

In the present study, eating undercooked meat was a major risk factor in the transmission of *T. gondii* infection which is in agreement with several studies conducted elsewhere (Koskiniemi *et al.* 1989; Lopez-Castillo *et al.*, 2005; Sroka *et al.*, 2010; Alvarado-Esquivel *et al.*, 2011). It is possible

that one of the sources of *T. gondii* infection in this study population is likely to be from eating meat with tachyzoites or bradyzoites hence leading to the infection transmission. Although in the current study eating in restaurants was more associated with a higher sero-prevalence of anti-*T. gondii* antibodies than those who did not, the difference was not statistically significant. However, some studies have demonstrated significant association between anti-*T. gondii* antibody seropositivity and the habit of eating in restaurants (Montoya & Liesenfeld, 2004).

There were no significant differences in sero-prevalence of anti-*T. gondii* antibodies in relation to tasting food while cooking, history of previous abortion, HIV status and being exposed to any kind of knowledge about toxoplasmosis. The absence of a significant relationship between the prevalence of *Toxoplasma* infection among pregnant women in Kigali and many of the factors explored in this study, does not rule out the possibility of these factors having some influence on the transmission of toxoplasmosis. Probably a larger study needs to be undertaken to explore further the role of the various risk factors for *T. gondii*.

From the findings of the present study, drinking untreated water and eating undercooked meat are the most important risk factors for *T. gondii* infection in Kigali. Further studies on the burden of maternal and congenital toxoplasmosis should be carried out in the country in order to advise policy on possible needs of instituting control programmes. Routine screening of *T. gondii* infections during antenatal care should be considered in Rwanda as the main strategy to minimize congenital toxoplasmosis.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EM conceived the study, collected the data and drafted the paper. JCSN, supported the data analysis and drafted the initial manuscript. KN, KJN, WJ supervised the execution of the study and edited the manuscript. All authors read and approved the final version of the manuscript.

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