Journal of the Egyptian Society of Parasitology, Vol. 47, No. 2, August 2017
J. Egypt. Soc. Parasitol. (JESP), 47(2), 2017: 381 - 388

SEROPREVALENCE OF *TOXOPLAMA GONDII* AMONG PRIMIGRAVIDA WOMEN AND THEIR NEONATES IN SOHAG GOVERNORATE, EGYPT

By

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Abstract

Infections with the *Toxoplasma gondii* parasite (*T. gondii*) occur worldwide and affect about one third of humanity. The study determined the prevalence of *T. gondii* among primigravidae Women and vertical transmission of *T gondii* in their newborns. Socio-demographic data and potential predisposing risk factors were studied using structured questionnaire. Venous blood samples (350 samples) were collected and tested for IgM & IgG anti-*Toxoplasma gondii* antibodies by enzyme-linked immunosorbent assay (ELISA) during pregnancy period and at birth for primigravidae and their neonates, PCR test used as confirmatory test for IgM positive samples. Of the 350 pregnant women included in the study 165 (47.1%) cases were IgG seropositive of them 33 (9.4) cases were IgM seropositive. 185 (52.9%) cases were seropositive for *Toxoplasma*-specific IgG and IgM antibodies. At birth serological study of primigravidae and their neonates revealed that 142 (40.5%) cases were chronically infected, 25 (7.1%) cases showed active infection and 183 (52.2%) cases were susceptible to infection; with prevalence of vertical transmission of 20% (5/25). Rural areas, contact with cats and ingestion of raw milk were statistically significantly associated with higher infection rates. Pigeon, sheep meat and ingestion of raw milk and egg represent the main source of infection in the studied area.

Keywords: *Toxoplasma gondii*, Primigravidae, Neonates, Seroprevalence, ELISA assay, PCR, Sohag, Upper Egypt.

Introduction

Toxoplasmamosis is a worldwide zoonotic disease (Dubey, 2010) and affect about one third of humanity (Hill *et al*, 2005). In pregnancy, the most common mechanisms of acquiring infection are through consuming raw or undercooked meats or contaminated water, or exposure to soil (gardening without gloves) or cat litter (Jones *et al*, 2001). Transfusion or organ transplantation from an infected person can also transmit the organism (Dubey and Jones, 2008). Most pregnant women (> 90%) with acquired T. gondii infection do not experience obvious signs and symptoms, and spontaneous recovery is the rule (Di Carlo *et al*, 2008). Only a small proportion will develop clinical signs of the disease (Kravetz and Federman, 2005). The clinical presentation in pregnant women is not more severe than in non-pregnant women, and most often occurs as an influenza-like illness (low-grade fever, malaise, lymphadenopathy), with an incubation period of 5 to 18 days following exposure (Stray-Pedersen, 1993). In immunocompromised pregnant women, T. gondii can cause severe encephalitis, myocarditis, pneumonitis, or hepatitis via acute infection or reactivation of a latent infection (Chen *et al*, 2005). Transmission to the fetus occurs predominantly in women who acquire their primary infection during pregnancy (Montoya and Remington, 2008). Maternal-fetal transmission occurs between one and four months following placental colonization by tachyzoites. The placenta remains infected for the duration of the pregnancy, and therefore may act as a reservoir supplying viable organisms to the fetus throughout pregnancy (Stray-Pedersen, 1993). Classic congenital toxoplasmosis is characterized by the tetrad described by Sabin in 1942: chorioretinitis, hydrocephalus, intracranial calcification and convulsion (Stray-Pedersen, 1993). Signs such as intracranial calcification, microcephaly, hydrocephalus, and severe intrau-

terine growth restriction strongly suggest in utero infection in the presence of documented maternal infection (Jones *et al*, 2003). Thus, dangerous effects of *T. gondii* on the young mothers especially the immunocompromised and the dangerous and chronic complications on the fetus.

This study was done to throw light on the prevalence and risk factors of *T. gondii* infection among primigravida and their neonates in Sohag Governorate which considered as a one of the remote areas of Egypt.

Subjects and Methods

This cross-sectional descriptive study was carried out on 350 women attending the Primary Health Care Units of Sohag Governorate for antenatal care and their neonates. The age of participants ranged from 18 to 35 years old. The idea of the present work was explained to the included women and written consent was taken from them. For each the Socio-demographic data (age, residence, educational level, type of housing (health or unhealthy according to WHO in Geneva 13-15 October, 2010), family income and potential predisposing risk factors (contact with cats, handling raw meat, consumption of raw milk and eggs) were studied using structured questionnaire through face-to-face interview. Inclusion Criteria were Primigravidae women, pregnancy confirmed by the clinician and by laboratory analysis with gestational age determined by the last menstrual period date and ultrasound. Maternal age ranged from 18-35years. The exclusion criteria were the non-consent to participate in the study.

Serological detection of *Toxoplasma gondii* infection: Venous blood samples (3ml) were taken from all primigravidae under complete sterilization during pregnancy, at birth and from their neonates; the collected samples were centrifuged at 3000 rpm for 15 minutes then the isolated sera collected, labeled and stored at -20°C until used (Garcia, 2007). The samples were tested for anti-*T. gondii* IgG & IgM antibodies with a commercially available enzyme linked immunoassay (*Toxoplasma* IgG & IgM; Diagnostic Automation Inc., Calabasas, CA, USA). Anti-*T. gondii* IgG antibody levels were expressed as International Units/ml, and a cut-off of ≥ 8 IU/ml was used for seropositivity. The cut-off for IgM seropositivity for each assay was obtained by multiplying the mean cut-off calibrator optical density by a correction factor (f = 0.35-0.40) printed on the label of calibrator according to the instructions of the manufacturer (positive and negative controls included in each run). To confirm the IgM positive results, a second blood sample (3ml) was immediately collected (from primigravidae and their neonates) and ELISA test was performed again accompanied by PCR as a complementary test. *T. gondii* was determined by real time PCR using a commercial kit (Primerdesign Ltd TM Genesig® (Advanced Kit, United Kingdom) Kit included *T. gondii* specific primer/probe mix, positive control template, internal extraction control primer/probe mix, internal extraction control DNA, endogenous control primer/probe mix, RNAse/ DNAse free water and template preparation buffer. *T. gondii* DNA was extracted from samples using genesig® Easy DNA/RNA extraction kit according to the manufacturers protocols. Briefly, template DNA was added to a reaction mixture containing 10ul of qPCR master mix, 1ul *T. gondii* specific primer/probe mix, 1ul internal extraction control DNA, 3ul RNAse/ DNAse free water, in a final volume of 15ul. For each DNA sample an endogenous control reaction was prepared by adding 10ul qPCR master mix, 1ul endogenous control primer/probe mix, 4ul RNAse/DNAse free water, in a final volume of 15ul. Then 15µl of each mix was pippeted into individual wells according to step one (applied biosystem) real-time PCR experimental plate set up. A reaction mix was prepared to calculate a standard curve for quantitative analysis of *T. gondii*. After adding uracil-Nglycosylase (UNG) in the reaction mix at 37°C for 15 min, initial activation of DNA polymerase at 95°C for 2min, denatur-

ation at 95°C for 10 seconds, then data collection at 60°C for 60 second. 50 PCR cycles were performed. The cycle threshold value (CT), indicative of the quantity of target gene at which the fluorescence exceeds a preset threshold, was determined. This threshold was defined as 20 times the standard deviation of the baseline fluorescent signal, i.e., the normalized fluorescent signal of the first few PCR cycles. After reaching the threshold, the sample was considered positive. During pregnancy primigravidae who confirmed to be infected received treatment with spiramycin (500mg/ three/day) for two months prescribed by the physicians on charge.

Statistical analysis: Data were tabulated and statistically analyzed by SPSS (version 16; SPSS Inc., Chicago, Illinois, USA). Descriptive statistics were used to summarize the data and the χ^2 -test was used. Differences were considered significant at P-values of 0.05 or less.

Results

In the present study, 205/350 primigravidae (58.5%) women were from rural areas and 145(41.5%) from urban ones. They were screened for *Toxo*-specific IgG & IgM antibodies; 132 (37.7%) cases were IgG seropositive (immune or chronically infected), 33(9.4%) cases were IgG & IgM positive indicated active infection (by ELISA & PCR) and 185(52.9%) cases were serongative (susceptible cases) for *Toxo*-specific IgG & IgM antibodies. At birth serological study of primigravidae women revealed 142 (40.5%) cases were chronically infected, 25 (7.1%) cases showed active infection (by ELISA and PCR) and 183 (52.2%) cases were susceptible to infection cases.

Serological study of newborns at birth revealed that 5 (1.4%) cases were positive for both IgG & IgM antibodies (active infection), 133(38%) cases were IgG-positive only (immune) and 212(60.6%) cases were negative for both. The prevalence of vertical transmission was 20% (5/25) and the index of vertical transmission represents 14.28 cases in 1000 live births (14.28/1000). Regarding the socioeconomic characteristics of the participants, there was a significant statistical association between IgG seroprevalence and age group (20-30) , rural residence, low level of education, unhealthy housing condition and low socioeconomic state among IgG seropositive compared to IgG seronegative primigravidae ($P < 0.05$).

The consumption of pigeon meat, or consumption of goat and sheep meat and ingestion of raw milk and eggs were the commonest among the IgG seropositive compared to IgG seronegative primigravidae and the differences were significant ($P > 0.05$). Also, the handling raw meat were more common among IgG seropositive group but the difference was non-significant ($p > 0.05$), without significant differences between groups regarding the gestational age and occupation ($p > 0.05$).

The details were given in tables (1, 2, 3 & 4)

Table 1: Seroprevalence of anit-*T gondii* IgG and IgM in primigravida during pregnancy and at birth.

Seroprevalence	During pregnancy N (%)	At birth N (%)	P-value
IgG +ve/IgM –ve	132 (37.7%)	142 (40.5%)	0.4
IgG +ve /IgM +ve	33 (9.4%)	25 (7.1%)	0.3
IgG -ve/IgM –ve	185(52.9%)	183 (52.2%)	0.8
Total	350	350	

Significant P <0.05

Table 2: Seroprevalence of *T. Gondii* using anti-*T gondii* IgG & IgM in neonates.

Seroprevalence	Num.	prevalence
IgG +ve/IgM –ve	133	38%
IgG +ve /IgM +ve	5	1.4 %
IgG -ve/IgM –ve	212	60.6%
Total	350	100%

Table 3: *T. gondii* seroprevalence in relation to socio-demographic data in primigravida pregnant women.

Characters	No. of subjects	Seropositive	Seronegative	P- value
Age: < 20 years	97	35 (36.1%)	62 (63.9%)	0.002
20- 30 years	231	124 (53.6%)	107 (46.4%)	
> 30 years	22	6 (27.3%)	16 (72.7%)	
Residence: Urban	145	53 (36.5%)	92 (63.5%)	0.00008
Rural	205	112 (54.6%)	93 (45.4%)	
Education: Illiterate	85	52 (61.1%)	33 (38.9%)	0.0001
Primary	135	71 (52.5%)	64 (47.5%)	
High school	96	33 (34.3%)	63 (65.7%)	
University	34	9 (26.4%)	25 (73.6)	
Gestational age: First trimester	71	31 (43.6%)	40 (56.4%)	0.5
Second trimester	124	56 (45.1%)	68 (55.9%)	
Third trimester	155	78 (50.3%)	77 (49.7%)	
Type of housing: Healthy	128	43 (33.6%)	85 (66.4.6%)	0.0001
Unhealthy	222	122 (54.9%)	100 (45.1.9%)	
Occupation: Employee:	166	73 (43.9%)	93(56.1%)	0.2
House wife	184	92 (50%)	92(50%)	
Socioeconomic level: High	9	1 (11.1%)	8 (88.9%)	0.00001
Medium	110	35 (31.8%)	75 (68.2%)	
Low	231	129 (55.8)	102 (44.2%)	

Significant P <0.05

Table 4: Seroprevalence of *T. gondii* in relation to risk factors in primigravida pregnant women.

Characters		No. of subjects	Prevalence of *T- gondii* infection		P value
			Seropositive (+ve)	Seronegative (-ve)	
Contact with cats and Handling raw meat:					
Contact with cats	Yes	162	93 (57.4 %)	69 (42.6%)	0.0003
	No	188	72 (38.3 %)	116 (61.7)	
Handling raw meat	Yes	112	57 (50.9%)	55 (49.1%)	0. 3
	No	238	108 (45.4%)	130 (54.6)	
Consumption of fresh meat:					
Pigeon meat	Yes	227	135 (59.5%)	92 (40.5%)	0.00001
	No	123	30 (24.4%)	93 (75.6%)	
Goat & sheep meat	Yes	152	39 (25.6%)	113 (74.4%)	0.00001
	No	198	126 (63.6%)	72 (36.4%)	
Chicken meat	Yes	345	164 (47.5%)	181 (52.5%)	0.2
	No	5	1 (20%)	4 (80%)	
Cattle meat	Yes	278	137 (49.3%)	141 (50.7%)	0.1
	No	72	28 (44.4%)	44 (55.6%)	
Raw milk & eggs ingestion:	Yes	159	92 (57.8%)	67 (42.2)	0.0002
	No	191	73 (38.2%)	118 (61.8%)	
Type of floor at home:					
Ceramic or wood		57	7 (12.3%)	50 (87.7%)	0.00001
Concrete		121	43 (35.5%)	78 (64.5%)	
Soil		172	115 (66.8%)	57 (33.2%)	

Significant P <0.05

Discussion

Toxoplasmosis is the third leading infectious disease cause of food-borne death after salmonellosis and listeriosis. Transmission to the fetus occurs congenitally (Montoya and Remington 2008). In the present study, 165 (47.1%) of primigravidae pregnant women were seropositive for anti-*Toxoplasma* IgG antibodies and 33 (9.4%) the seropositive for the anti-Toxoplasma gondii IgM antibodies. These results agreed with other Egyptian studies. Tammam *et al*. (2013) in Qena Governorate reported anti-*T. gondii*-IgG in thewomen with early spontaneous miscarriage 46.1%. Hussien *et al*. (2001) in Qualyobia Governorate reported anti-*T. gondii*-IgG 44.7% among aborted women. El Deeb *et al*. (2012) in Menoufia Governorate reported higher IgG (67.5%) and Ibrahim *et al*. (1997) in Gharbia Governorate reported (52.4%). Lower seroprevalence were reported by Egypt; Aboelhadid *et al*. (2013) in Beni Suef Governorate reported (35.2%), in Dakahlia Governorate Aboul-

Hassan et al. (1997) reported (23.85%) and Ghoneim *et al*. (2009) in El- Fayoum Governorate reported (30.5%). In Arab countries, toxoplasmosis seroprevalence among women was 37 in Jordan % (Morsy and Michael, 1980), 58.2% in Kuwait (Al-Nakib *et al*, 1983), 37.5% in Libya (Kassem and Morsy, 1991) and 50.6% in Morocco **(**ElMansouri *et al*, 2007)

Abroad, higher anti-*Toxo*-IgG seroprevalence (68.37%, 51.8% & 54%) was reported in Brazil, Kenya and Iran by da Silva *et al*. (2015), Griffin and Williams (1983) and Assmar *et al*. (1997) respectively. In Iran, IgG seroprevalence (29%) reported by Tabatabaie *et al*. (2015) was lower than the present result. Differences in seroprevalence of the specific anti-*T. gondii* IgG antibodies could be related to environmental, climatic and/or low socioeconomic conditions.

On the other hand, the present work revealed that seroprevalence of IgM in the primigravidae was 9.4%. Others reported higher IgM seroprevalence; (18.4%) in Qena by Tammam *et al*. (2013), (23.8%), in Qualyobia by Hussien *et al*. (2001), and (20.45% & 7.95%) among pregnant and non-pregnant women respectively in El Fayoum by Ghoneim *et al*. (2009). Saleh *et al.* (2014) in Alexandria reported 22.2% among pregn- ant women & 20% among non-pregnant ones antibodies against *T. gondii*. Abroad, low seroprevalence was 2% in Brazil (da Silva *et al*, 2015) and 5.33% in Iran (Assmar *et al*, 1997).

In the present study, the *Toxoplasma*-specific IgG & IgM antibodies in the neonates were 133 (38%) and 5(1.4%) respectively; the prevalence of vertical transmission was 5/25 (20%). Higher prevalence of vertical transmission was reported in Brazil (28%) by da Silva *et al*. (2015). On the other hand, lower prevalence of vertical transmission (less than 1%) was reported in rural Egyptian area by Ahmed *et al*. (1996).

In the present study, the index of vertical transmission represent 14.28 cases in 1000 live births (14.28/1000) which agreed with da Silva *et al* (2015) in Brazil who reported 14.37 cases in 1000 live births. In the present study prenatal antibiotic therapy of toxoplasmosis during pregnancy had no impact on the feto-maternal transmission rate. This result agreed with Foulon *et al.* (1999) who reported that treatment of toxoplasmosis during pregnancy had no impact on the feto-maternal transmission rate but reduced the rate of sequelae among the infected infants. In the present study, there was association between maternal age and seropositivity of *T. gondii*, it was higher in the age group 20-30 years and lower above 30 years. Studies in Iran, Venezuela and Croatia reported that the peak age was >30 and <15 years respectively (Fallahi *et al*, 2009; Diaz-Suárez and Estevez, 2009). Others reported no association between maternal age and seropositivity of *T. gondii* (da Silva *et al*, 2015).

In the present study, rural residence women showed higher anti-*Toxo*-IgG positivity (54.6%) than the anti-*Tox*-IgG negative ones (45.4%). The present results agreed with Tammam *et al*, (2013) in Qena who reported that *T. gondii* infection was 3.8 times greater in individuals in rural areas than those in urban areas. But, Cosme *et al*, (2014) reported higher seropositivity in urban than in rural areas. This may be due to direct contact with domestic animals, poor environmental sanitation, unwashed food and low education level as well as abundance of stray dogs and cats.

In Egypt, Rifaat *et al*. (1967) reported natural *Toxoplasma* infection in the insectivorus animals, and Rifaat *et al.* (1969) reported toxoplasmosis seropositivity in chi-cken and pigeons and farm animals (Rifaat *et al*, 1977), as well as camels (Hilali *et al*, 1998). Khaled *et al.* (1982) reported toxoplasmosis seropositivity in stray dogs. Haridy *et al*. (2009) reported toxoplasmosis seropositivity in the draught horses as well as donkeys and donkey's milk (Haridy *et al*, 2010). Besides, Morsy *et al.* (1987) reported sero-toxoplasmosis in commensal and wild rodents.

On the other hand, Al-Qurashi (2004) in

the rural areas in eastern region of Saudi Arabia reported 26% among both sexes and increased with the increase of ages and was higher in the housewives, employees and farmers than in the students and children.

In the present study, the data analysis showed significant relationship between the rate of infection and low level of education, unhealthy housing and low socioeconomic level, meanwhile no significant relationship were found with occupation and the gestational ages of primigravidae. Saleh *et al.* (2014) in Egypt found that the health education about the toxoplasmosis should be tailored to women whether married or single to help them in avoiding the risk of infection. They added that frequent periodic *Toxoplasma* serodiagnosis should be done for people who continuously contact with the cats. Also, they added that adherence to strict infection prevention measures is a must to eliminate exposure to toxoplasmosis infection. Tabatabaie *et al.* (2015) in Iran reported no significant relationship between infection rate and gestational age and education

In the current study, there was a significant association between IgG seroprevalence and contact with the cats, eating of pigeon meat, consumption of goat meat, ingestion of raw milk and eggs as well as the types of house floor ($P < 0.05$), meanwhile no significant relationship were found with cattle ,chicken meat consumption and raw meat handling. Cosme *et al.* (2014) in Mexico reported no significant relationship between IgG seroprevalence and contacts with cats, consumption of pigeon meat, chicken meat, goat meat and type of floor meanwhile consumption of turkey meat showed significant relationship ($P < 0.05$).

Nevertheless, studies from Taiwan (Lin *et al*, 2008); Ethiopia (Zemene *et al*, 2010) and Egypt (Al-Kappany *et al*, 2010) showed a significant association between contact with the cats and seroprevalence of *T. gondii*. In cats, the bradyzoite replicates slowly, exhibit's the low immunogenicity, and partly protected the host from the parasite-induced cell rupture and immunopathology (Elsheikha and Morsy, 2009).

Conclusion

The seroprevalence of *T. gondii* among the pregnant women and their neonates in Sohag Governorate is high, with a significant proportion of pregnant women and their neonates at the risk for contracting the *T. gondii* infections. The rural residence, unhealthy housing and contact with cats play important role in magnification of infection so antenatal screening and educational programs are needed.

In the studied area, consumption of pigeon and goat meat represented the source of *T. gondii* infection so more veterinary studies needed for control of infection. Regimen of *Toxoplasma gondii* treatment faced some problems with the positive cases and more studies needed to put more efficient one.

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