

## SEROPREVALENCE AND RISK FACTORS OF *TOXOPLASMA GONDII* INFECTION AMONG PREGNANT WOMEN IN AD-DAWADIMI GENERAL HOSPITAL, KINGDOM OF SAUDI ARABIA

By

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### Abstract

The present study investigated the seroprevalence of two antibodies (anti-*T. gondii* IgG and IgM) among the pregnant women in Ad-Dawadimi City, along with possible risk factors associated with *Toxoplasma gondii*. A total of 461 pregnant women were enrolled at various stages of pregnancy, either asymptomatic or having clinical signs and symptoms suggestive of toxoplasmosis (fever, lymphadenopathy, hepatomegaly, splenomegaly, and rash). A short face to face questionnaire interview for pregnant women was carried out to obtain information regarding *T. gondii* infection. Out of the 461 blood samples tested, 189 (40.9%) were seropositive for specific anti-*T. gondii* IgG antibodies. But, none of the women had positive IgM results. Seroprevalence of *T. gondii* antibodies was high among pregnant women and the prevalence showed a corresponding increase as the age of the pregnant women increases. This study shows that Toxoplasmosis is an endemic disease in Ad-Dawadimi city. Thus, an awareness creation program on the modes of transmission and prevention of *T. gondii* among women of child bearing age in general and pregnant women in particular should be organized during the antenatal care follow up.

**Key Words:** Saudi Arabia, Ad-Dawadimi City, *Toxoplasma gondii*, Seroprevalence, ELISA.

### Introduction

Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*. It is commonly transmitted to humans by the accidental ingestion of the infective stage in the contaminated soil and drinking water, and consumption of infected raw meat. It can also be transmitted congenitally during the pregnancy (Dubey, 2010). Primary infections with *T. gondii* acquired during pregnancy are usually asymptomatic for the pregnant woman, but can lead to serious neonatal complications (Flatt and Shetty, 2013). Toxoplasmosis in pregnancy has been associated with miscarriage, hydrocephalus, cerebral calcification and chorioretinitis in the newborn. Primary infection with *T. gondii* during the third trimester of pregnancy carries a higher risk of congenital transmission than if it is acquired

during the first trimester (Al-Hamdani and Mahdi, 1997; Dunn *et al*, 1999). Generally, it is estimated that about one third of the world's population is infected with *T. gondii*. In developed countries, congenital toxoplasmosis affected 0.01% - 0.1% of infants (Montoya and Remington, 2008).

*Toxoplasma gondii* seroprevalence is evolving worldwide and is subjected to complex environmental, socioeconomic and health-related practices. The high prevalence of the infection was reported among the pregnant women and women of childbearing age from different foci in Latin America, parts of Eastern, Central Europe, the Middle East, parts of Southeast Asia and Africa (Pappas *et al*, 2009). However, the prevalence of *T. gondii* in pregnant women in China was less than 10% (Gao *et al*, 2012). In Africa, over-

all seroprevalence rate as high as 92.5% was reported (Ayi *et al*, 2009). In Saudi Arabia, the *T. gondii* infection among pregnant women varied in the different region, For example, it was 21.5% in Riyadh (Shoura *et al*, 1973), 42.1% in Dammam (Abbas *et al*, 1986), in Abha 31.6% (El-Hady, 1999), 25% in Jeddah (Tonkal, 2009), in AlAhsa (Al-Mohammed *et al*, 2010) 32.3% and in Najran (El-Shahawy *et al*, 2014). Undoubtedly, the early and proper diagnosis of infection in pregnant women (with the possible risk of transplacental transmission) or their babies leads to effective treatment and minimizes complications (Nagaty *et al*, 2009). During the acute course of infection, *Toxoplasma* antigen in serum and other body fluids could be detected by the widely accepted especially for latent antibody (Turunen, 1983; Remington and Desmonts, 1990). The ELISA assay detected the total immunoglobulins (Carlier *et al*, 1980; Ahlfors, 1989; Konishi, 1989; Suzuki *et al*, 1989; Al-mohammed, 2011). Pregnant women have a great vulnerability to *T. gondii* due to the alterations in the immune mechanisms inherent to gestation.

The present study aimed at the investigation of anti-*Toxoplasma gondii* IgG & IgM among pregnant women in Ad-Dawadimi City, Saudi Arabia along with discussion of the possible risk factors associated with toxoplasmosis.

### **Subjects and Methods**

Study site: Ad-Dawadimi City is located on top of Najd hill, the central area of Saudi Arabia, about 280 Kilometers West of Riyadh, the capital City distinguish with geographical position at latitude 24° North and longitude 44° East. The population is about 240,000 thousand people (Fig. 1).

Data Collection: A prospective study was carried out at the Obstetrics & Gynecology Clinic at Ad-Dawadimi General Hospital (Ministry of Health, Saudi Arabia) during the period from May 2014 to December 2016. The study protocol was approved by the Scientific Research Committee, Shaqra

University. The study sample size was calculated based on the sero-prevalence of *T. gondii* IgG & IgM in Saudi Arabia (Sarwat *et al*, 1993; Abdulla *et al*, 1994; Al-Amari, 1994; El-Hady, 1999). A total of 461 pregnant women with different ages were enrolled at pregnancy various stages, either asymptomatic and/or with suggestive signs and (miscarriage, fever, lymphadenopathy, hepatomegaly, splenomegaly, rash...etc.). The patients with history of anti-*Toxoplasma* treatment during pregnancy were excluded. Informed consent was taken from each patient. A short face to face questionnaire interview was carried out to obtain information regarding *T. gondii* infection including age, nationality, residential address, consumption of raw or undercooked meat products, keeping pets such as cats, known previous history of toxoplasmosis. Additional information about the number of pregnancies, children and abortion was also collected.

Serodiagnosis of *T. gondii*: Approximately 5ml of venous blood was aseptically drawn from each woman. Sera were tested for anti-*T. gondii* antibodies using ELISA kit for direct IgM antibody detection (REF 51119, Human GMBH, Wiesbaden Germany, www.human.de), the microtitre wells were coated with anti-human IgM antibodies (mouse) and Human Toxo-IgG ELISA, REF 51209) following the manufacturer's instructions.

Statistical analysis: Data were coded, validated and analyzed using STATA version 12 (College Station, Texas, USA). Categorical variables were summarized as proportions and analyzed using the Pearson's Chi-square test to present the difference among groups. Continuous variables were as mean standard deviation ( $\pm$ ). Univariate analysis and multivariate logistic regression models were fitted to determine factors associated with infection (age, residence, occupation, education level, gravidity, eating undercooked meat and contact with cats). A backward-stepwise selection model was used; risk factors with a P value less than 0.2 fitted into multivariate

logistic regression analysis. Odds ratios (OR) and their 95% confidence interval [95% CI] were noted. Factors with P value less than 0.05 on multivariate logistic regression analysis were considered to have a significant association with *T. gondii*.

### Results

A total of 461 pregnant women were enrolled with mean age of 26.3±6.4 years with majority of women 192/461 (41.6%) aged between 16-25 years. Majority were from an urban residence 277/461 (60.08%), housewi-

ves 300/461 (65.07%), multigravida 203/461 (44.3%) and on the third trimester 218/461 (47.2%). Majority 259/461 (56.18%) had primary education and 160/461 (34.7%) attained more education level (Tab. 1). Of 461 pregnant women, 189 (40.9%) were anti-*T. gondii*-specific IgG antibodies positive, indicated past infection (Tab. 2). None had positive IgM. *T. gondii*-specific IgG antibodies were higher in pregnant women in urban areas (46.5%) than in rural ones (32.6%) [OR=2.4, 95% CI: 1.3-3.9, p=0.001].

Table 1: *T. gondii* along with demographic characteristics of pregnant women in Ad-Dawadimi City.

Demographic Characteristics	<i>T. gondii</i> (seroprevalence N= 461)		Total
	Positive	Negative	
Age (years) 16-25	61(32.10)	129(67.9)	190
26-35	77(44.76)	95(55.3)	172
36-45	51(51.61)	48(48.4)	99
Residence: Urban	129(46.5)	148(53.5)	277
: Rural	60(32.6)	124(67.4)	184
Education: Illiterate	19(45.23)	23(54.7)	42
: Primary	97(37.45)	162(62.5)	259
: 10 <sup>th</sup> grade or more	73(45.62)	87(54.3)	160
Occupation: Housewives	117(39)	183(61)	300
: Employed	48(44)	61(56)	109
: Business	23(44.2)	29(55.8)	52
Gravidity: Primigravid	53(32.7)	109(67.2)	162
: Multigravid	71(34.9)	132(65.02)	203
: Grand multigravid	27(28.15)	69(71.8)	96
Trimester: 1 <sup>st</sup>	8(21.6)	29(78.3)	37
: 2 <sup>nd</sup>	77(37.3)	129(62.6)	206
: 3 <sup>rd</sup>	104(47.7)	214(52.2)	218

Table 2: Factors associated with *T. gondii* infection among pregnant women (N=461) in Ad-Dawadimi city.

Characteristics	Toxoplasma sero-prevalence		Univariate analysis	Multivariate analysis
	positive	negative		
Age (years): 16-25	61(32.1%)	129(67.9%)	1	1
: 26-35	77(44.7%)	95(55.3%)	2.3 [1.4-3.8] <0.001	2.1 [1.4-3.6] <0.001
: 36-45	51(51.5%)	48(48.4%)	2.5 [1.1-6.1] <0.036	2.2 [0.8-5.4] <0.076
Residence: Urban	129(46.5%)	148 (53.5%)	1	1
: Rural	60(32.6%)	124(67.4%)	2.4 [1.5-3.9] <0.001	2.1 [1.3-3.7] <0.001
Occupation: Housewives	117(39.3%)	183(60.7)	1	1
: Employed	48 (44.0%)	61(56.0%)	1.8 [1.1-3.0] <0.005	
: Business	23(44.2%)	29(55.8%)	1.9 [1.2-2.9] <0.006	
Education: Illiterate	19(45.2%)	23(54.7%)	1	1
: Primary	97(37.4%)	162(62.5%)	0.8[0.2-2.0] <0.545	
: Secondary/more	73(45.6%)	87(54.3%)	1.3 [0.5-3.8] <0.480	
Gravidity: Prim gravid	53(32.7%)	109 (67.2%)	1	
: Multi gravid	71(34.9%)	132(65.02%)	1.2[0.6-1.9] <0.580	
Grandmulti gravid	27(28.1%)	69(71.8%)	1.1 [0.5-2.2] <0.925	
Eating under cooked meat: Yes	26(52%)	24(48%)	1	
: No	163(39.6%)	248(60.4%)	1.6[0.8-2.2] <0.385	
Contacts with cat: No	16(59.2%)	11(60.4)	1	
: Yes	173(39.8%)	261(60.2)	0.8[0.5-1.4] < 0.481	

A total of 300/461 (65.07%) were housewives, of whom 39.3% (118/300) were posi-

tive for anti-*T. gondii*-antibodies. On the univariate analysis, there was a significant

difference in the *Toxoplasma* sero-positivity among women with different occupations; employed/business pregnant ones had a higher sero-positivity rate of *T. gondii*-specific antibodies than housewives (44.1% vs. 39.3%) (OR=1.7, 95% CI: 1.1-3.0, p=0.005). The occupation factor of pregnant women was not subjected to multivariate analysis because it had a co-linearity relation with residence; as most of the housewives were living in urban areas.

### Discussion

The study showed an overall 40.99% seroprevalence of anti-*T. gondii* antibody among pregnant women in Ad-Dawadimi City. This result was lower than the prevalence among the general population reported from Jimma town, Southwestern Ethiopia, where 83.6% of the sampled population had evidence of *T. gondii* infection (Zemene *et al.*, 2010).

Besides, IgG seroprevalence of *T. gondii* obtained in this study was similar to those reported from Palestine, Saudi Arabia, Brazil, Morocco and China (Nijem 2009; Al-Mohammad *et al.*, 2010; Vaz *et al.*, 2010; ElMansouri *et al.*, 2007; Hao, 2013). In contrast, lower seroprevalence of *T. gondii* was reported in many European Countries and the United States of America (Dunn *et al.*, 1999). This wide variability could be attributed to differences in climatic conditions and personal hygienic practices, feeding habits, socio-economic and literacy status of studied subjects. The prevalence of 40.99% was much higher than the results reported in Saudi Arabia, as in Abha 31.6% (El Hady, 1999), in Makkah 35.6% (Ghazi *et al.*, 2002); in Najran 32.3% (El-Shahawy *et al.*, 2014), in Jazan Province 24.1% (Aqeely *et al.*, 2014) and in Southwestern 38.8% (Mona *et al.*, 2014). Higher prevalence rates were also reported in other neighboring Arab countries as 37% in Jordan (Morsy and Michael, 1980), 58.2% in Kuwait (Al-Nakib *et al.*, 1983), 37.5% in Libya (Kassem and Morsy, 1991). Nevertheless, the present rate (40.99%) was more than that in Egypt 22.2% in pregnant nurses & 20% in non-

pregnant (Saleh *et al.*, 2014). On the other hand, in Riyadh, Kandil *et al.* (1979) reported *Toxoplasma* seroprevalence of 18% among healthy parous women and Al-Meshari *et al.* (1989) in King Khalid Hospital reported anti-*Toxoplasma*-IgG in 380 pregnant mothers. Morsy *et al.* (1994) in Riyadh District reported anti-*Toxoplasma* antibodies in the abundant commensal rodents (*Mus musculus* and *Rattus norvegicus*) and Elamin (2014) identified the *T. gondii* genotypes of *Rattus rattus* and added that the better understanding of *T. gondii* pathogenesis pave the way to disease control.. Also, Alanazi (2013) reported anti-*T. gondii* antibodies in 36.4% (325/891) sheep and 35.3% (196/555) goats and 23.6% (43/182) camels. He added that the zoonotic animal toxoplasmosis had its bad input economy and welfare. Elsafi *et al.* (2015) in Dhahran, among pregnant women reported IgG and IgM against *T. gondii* as 28.5% & 3% respectively. Besides, Alonso *et al.* (1984) stated that patients with AIDS developed up to 50% cerebral toxoplasmosis. Al-Harthi *et al.* (1988) in Saudi Arabia reported a case of cerebral toxoplasmosis. The specific IgG antibodies rising titer and/or specific IgM antibodies are the best indicator of active infection (Barsoum, 2006), but might need to have more than one sample from the patient. Moncada and Montoya (2012) stated that more than a third of the world's population has been infected with the parasite, but the seroprevalence was unevenly distributed across countries and different socioeconomic strata congenital toxoplasmosis could be prevented and treated during gestation. Less severe disease is commonly reported in countries where prenatal screening and treatment were systematically implemented.

In the present study, the business women and employed pregnant women had higher infection rates with *T. gondii* than housewives, in contrary to other studies (Ayi *et al.*, 2009; Sroka *et al.*, 2010). This may partly be explained by the economic status of women where employed and business women have

to increase family income and live in urban areas and depended on the already processed take away food compared to housewives live in rural areas and depended on homemade food (Al-Nakib *et al*, 1983; Ghazi *et al*, 2002). This agreed with Hao (2013) in China who found that residents in the urban areas were more infected with *T. gondii* than those in the rural ones. Also, Al-Qurashi (2004) in rural areas in eastern region of Saudi Arabia reported 26% among both sexes but increased with age and was higher in housewives, employees and farmers than in students and children. He added that not all rural areas were less than urban ones; so long there is good rural health care.

Consumption of raw contaminated fruits and/or undercooked meat was reported as potential sources of *T. gondii* infection (Sroka *et al*, 2010). Moreover, Amin and Morsy (1997) in Jeddah municipal abattoir reported anti-*Toxoplasma* antibodies in butchers and slaughtered sheep and goats. On the other hand, abroad among those eating pork, Abdulmawjood *et al*. (2014) reported *T. gondii* in raw sausages using in-house developed and that *T. gondii* survived in raw-sausage-manufacturing-process including the different ripening processes. Also, blood transfusion and needle-stich injury must be considered (Saleh *et al*, 2017). Contact with cat-litter may pose another risk for *T. gondii* infection. In the present study no significant association was between *T. gondii* infection and a history of cat contact. The findings were consistent with studies done in Palestine (Al-Nakib *et al*, 1983), Turkey (Ghon-eim *et al*, 2009) and Nigeria (Ishaku *et al*, 2009). Nevertheless, studies from Ethiopia (Zemene *et al*, 2010) and Taiwan (Lin *et al*, 2008) showed a significant association between contact with cats and seroprevalence of *T. gondii*.

In Egypt, Al-Kappany *et al*. (2010) isolated 115 viable *T. gondii* isolates from cats' tissues and genotyped by 10 PCR-restriction fragment length polymorphism markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-

8, c29-2, L358, PK1, and Apico) and DNA from tachyzoites. They concluded that a strong clonal population structure with dominance of clonal Type II & III lineages of *T. gondii* in Egyptian feral cats. Al-Kappany *et al*. (2011) reported a high prevalence of *T. gondii*, *Bartonella* spp., and FIV infections in cats and added that cats were important in the epidemiology of *T. gondii*, as being the only hosts that excrete environmentally resistant oocysts in feces. In the definitive host or cats, the bradyzoite replicates slowly, exhibits low immunogenicity, and partly protects the host from parasite-induced cell rupture and immunopathology (Elsheikha and Morsy, 2009). However, the risk of contracting *T. gondii* infection may not be just the mere contact with cats, but the way the cats' litter handling.

### **Conclusion**

Seroprevalence of *T. gondii*-specific antibodies is high among pregnant women in Ad-Dawadimi. Advanced woman's age, urban residence and being an employed or a business woman were the independent risk factors associated with the presence of *T. gondii* infections. Therefore, an awareness creation program on the modes of transmission and prevention of *T. gondii* among women of child bearing age in general and pregnant women in particular should be organized during the antenatal care follow up. This study may also be informative and useful to the public health community.

### **Recommendations**

The health education about toxoplasmosis must be tailored to women whether married or single to help in avoiding the risk of toxoplasmosis. People owners' pet-cats must be periodically sero-examination. An intervention must be implemented for the successful improvement in knowledge of the toxoplasmosis control measures. Treating livestock influence the human toxoplasmosis risk.

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Fig. 1: Ad-Dawadimi location with respect to Riyadh Province