

Seroprevalence and risk factors of *Toxoplasma gondii* infection among pregnant women in south western, Saudi Arabia

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Abstract This study aimed to determine the seroprevalence of *Toxoplasma gondii* in pregnant women in the south western region of Saudi Arabia and to find out the possible risk factors that may lead to infection. This cross sectional hospital based study was carried out at three hospitals in the south western region of Saudi Arabia from January 2008 to August 2010. Blood samples from 487 pregnant women were collected and used to detect anti-*T. gondii* antibodies IgM and IgG by enzyme linked immunosorbent assay (ELISA). A questionnaire interview was carried out to ask about some risk factors of infection. Among the 487 studied pregnant women, 38.8 % were seropositive for anti *T. gondii* IgG while 6.2 % were positive for anti *T. gondii* IgM and 3.3 % were positive for both anti *T. gondii* IgG & IgM. The only risk factor associated with seropositive anti *T. gondii* IgM was the history of the intake of immunosuppressive drugs. Regarding anti *T. gondii* IgG seropositivity, it was found to increase significantly with increased age, number of

gravida and parities, and previous history of toxoplasmosis. The seroprevalence of *T. gondii* IgG & IgM by ELISA among pregnant women in the south western region of Saudi Arabia is considerable with few identifiable significant risk factors reported.

Keywords *Toxoplasma gondii* · ELISA · Seroprevalence · Risk factors · Pregnant women

Introduction

Toxoplasma infection is often benign, yet congenital toxoplasmosis can lead to severe sequelae for the foetus and the newborn. Mother-to-child transmission depends on the gestational age. When infections occur soon before or during the first trimester of pregnancy it may cause miscarriage, death in utero, or severe neurological lesions. Whereas fetal infection occurring later in pregnancy may result in either congenital disease or sub-clinical infection (Remington et al. 2005).

Toxoplasma gondii seroprevalence is evolving worldwide and is subjected to complex environmental, socio-economic and health-related practices (Pappas et al. 2009). It is more prevalent in some regions than in others and varies between different population groups within the same region (Asthana et al. 2006). In Saudi Arabia, the seroprevalence among pregnant women varies according to the region from 25 % in Jeddah (Tonkal 2008) to 42.1 % in Dammam (Abbas et al. 1986).

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). Serological diagnosis represents the first and most widely used

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approach to define the stage of infection, whether current, recent or past (Sensini 2006).

Pregnant women have a great vulnerability to *Toxoplasma* due to the alterations in the immune mechanisms inherent to gestation. So, this study is of the utmost importance to determine the immunity of pregnant women in the south western region to this parasite. Provided with this, we can estimate the magnitude of toxoplasmosis among this critical group. This study will also help to define the possible risk factors that may lead to toxoplasma infection in order to develop extensive health education campaigns to increase the awareness and the knowledge of preventive behavior among Saudi population toward toxoplasmosis.

Materials and methods

Study population

This cross sectional hospital based study was conducted in three hospitals: (a) King Faisal Armed Forces Hospital (KFAFH) in Khamis Mushait, (b) Abha General Hospital (AGH) and (c) Maternal and Child Hospital (MCH) in Abha, south western region of Saudi Arabia from January 2008 to August 2010. A total sample of 487 pregnant women attending the antenatal clinics of the three hospitals was enrolled randomly in the study.

An approval was taken from the Ethical Committee of King Khalid University and the previously mentioned hospitals to conduct the study. An informed written consent of the pregnant women who accepted to participate in the study was secured.

Questionnaire interview on *T. gondii* infection

A short face to face questionnaire interview for pregnant women was carried out in the antenatal clinics of the three hospitals to obtain information regarding *T. gondii* infection including age, residential address, consumption of raw or undercooked meat products, drinking unboiled milk, keeping pets including cats, intake of immunosuppressive drugs, known previous history of toxoplasmosis, and history of blood transfusion or organ transplantation. Additional information about number of pregnancies, children, abortion, and ultrasonographic follow-up was collected.

Serological detection of *T. gondii* infection

From each pregnant woman, five ml of blood was withdrawn upon the first visit to the antenatal clinic to determine serum IgM and IgG antibody (Ab) levels against *T. gondii* antigens using the TOXO IgM μ -capture enzyme

linked immunosorbent assay (ELISA) for direct IgM antibody detection (REF 51119) (Human GMBH, Wiesbaden Germany, www.human.de) in which the microtitre wells were coated with anti-human IgM antibodies (mouse) and the HUMAN TOXO IgG ELISA (REF 51209) (Human GMBH, Wiesbaden Germany, www.human.de) which is based on the classical ELISA technique. The microtitre strip wells were coated with *Toxoplasma* antigens prepared from sonicated whole *T. gondii* parasites (ELISA). Methodology was done as described by the manufacturer. Equivocal results were supposed to be verified 2–3 weeks later to be considered as positive or negative, but this was not done and were excluded.

Statistical analysis

Data were coded, validated and analyzed using SPSS PC+ version 13 software package. Frequency, percentage, arithmetic mean, median and mode were used to present the data. Chi square and Student “*t*” test were used as tests of significance at 5 % level of significance.

Results

The present study included a total sample of 487 pregnant women recruited from three hospitals. Most of them were from King Faisal Armed Forces Hospital (252, 51.7 %) followed by Abha General Hospital (184, 37.8 %) and the Maternal and Child Hospital (51, 10.5 %).

Socio-demographic profile

Table 1 shows the age of pregnant women in the study sample ranged from 16 years to more than 41 years. The average age was 28.6 ± 5.5 years and the median age was 28 years. The majority of the study sample were housewives (400, 82.1 %). Only 87 women were working, the majority of them were teachers (63, 12.9 %).

Obstetric history

The average number of pregnancies was 3.6 ± 2.8 with a median of three pregnancies. The most frequent gravida history was 2–4 pregnancies in 41.3 % (201/487) of the pregnant women. Regarding “parity” of the studied sample, the average number of deliveries was 2.1 ± 2.3 with a median of 1 delivery. The most frequent “parity” history was 2–4 deliveries (177, 36.3 %). The gestational age ranged from 4 to 39 weeks with an average of 21.2 ± 8.7 weeks and a median of 20 weeks. More than half of the study sample were recruited during the second trimester of pregnancy ($n = 271$, 55.6 %). Two-thirds of

Table 1 Socio-demographic profile and obstetric history of the study sample

Socio-demographic profile and obstetric history	N (n = 487)	%
(1) Age groups (years)		
16–19	14	2.9
20–30	323	66.3
31–40	143	29.4
≥41	7	1.4
(2) Occupation		
Housewife	400	82.1
Teacher	63	12.9
Student	12	2.5
Nurse	8	1.6
Governmental employee	4	0.8
(3) Gravida		
1	141	29
2–4	201	41.3
≥5	145	29.8
(4) Parity		
Zero	166	34.1
1	79	16.2
2–4	177	36.3
≥5	65	13.3
(5) Gestational age		
First trimester	94	19.3
Second trimester	271	55.6
Third trimester	122	25.1
(6) Abnormal previous pregnancy outcomes:		
Abortions		
0	325	66.7
1–2	142	29.2
≥3	20	4.1
Malformed fetus		
Yes	7	1.4
No	480	98.6

the sample had no history of abnormal pregnancy outcomes in the form of abortions ($n = 325$, 66.7 %) while most of it did not give birth to a malformed fetus (480, 98.6 %) (Table 1).

Past history of toxoplasma infection and exposure to risk factors

Around 97 % had neither previous history of toxoplasmosis, nor did they give history of intake of spiramycin to treat toxoplasmosis. The majority of the pregnant women (97–100 %) had no history of exposure to different risk factors.

Seroprevalence of “anti-*Toxoplasma*” antibodies among pregnant women

The seroprevalence of “Anti-*Toxoplasma*” antibodies is shown in Table 2. The seroprevalence of anti-*Toxoplasma* IgG & IgM was found to be 38.8 % (189 out of 487) and 6.2 % (30 out of 487), respectively. Combined anti *Toxoplasma* IgG & IgM antibodies were found in 3.3 % (16 out of 487).

Determinants of sero-positive “*Toxoplasma gondii*” IgM among pregnant women

Table 3 shows that the IgM anti *T. gondii* seropositivity was higher although not statistically significant among pregnant women aged 16–19 years, housewives, and among those who were pregnant for 2–4 times.

Moreover, the anti *T. gondii* IgG seroprevalence was found to increase significantly with age ($\chi^2 = 7.97$, $P = 0.048$), with increased number of pregnancies ($\chi^2 = 5.70$, $P = 0.05$) and with the increased number of parity ($\chi^2 = 7.99$, $P = 0.046$). On the other hand, gestational age, previous history of abortions or malformed fetuses, was of no significant value in developing seropositivity to *T. gondii* IgG.

Table 4 shows that the seroprevalence of anti *T. gondii* IgM was significantly higher among pregnant women who had a history of intake of immunosuppressive drugs ($P = 0.03$). On the other hand, other risk factors were of no statistical significant value in developing *T. gondii* IgM. Previous history of toxoplasmosis was significantly associated with the development of anti *T. gondii* IgG seropositivity ($\chi^2 = 3.94$, $P = 0.047$) while there were no significant associations with other risk factors.

Discussion

Although toxoplasmosis is considered harmless for non-pregnant women, it is potentially harmful during pregnancy, especially at the first trimester (Giannoulis et al. 2008). In the current study, the seropositivity for anti *T.gondii* IgG & IgM was 38.8 % (189/487) and 6.2 % (30/487), respectively. The combined seropositivity for anti *T. gondii* IgG and IgM was found to be 3.3 % (16/487) using ELISA.

Compared to the outcomes of similar studies done in different regions of Saudi Arabia, the seroprevalence of *T. gondii* IgG is in agreement with that reported in a recent Saudi study in Riyadh in which anti *T. gondii* IgG was found in 38 % of the studied pregnant women (Almogren 2011). In addition to that, anti-*Toxoplasma* IgG seropositivity was reported in the Eastern province to be 39.4 % (69/175)

Table 2 Seroprevalence of anti-Toxoplasma antibodies (IgM & IgG) among pregnant women

Anti-toxoplasma IgM	Anti-toxoplasma IgG				Total	
	Negative		Positive		N	%
	N	%	N	%		
Negative count	260	53.4	158	32.4	418	85.8
Positive count	14	2.9	16	3.3	30	6.2
Equivocal count	24	4.9	15	3.1	39	8
Total	298	61.2	189	38.8	487	100

Table 3 Seroprevalence of *T. gondii* IgM and IgG antibodies in pregnant women on admission by age and occupation and obstetric history

Variable	<i>T. gondii</i> IgM					<i>T. gondii</i> IgG				
	Negative		Positive		P	Negative		Positive		P
	N	%	N	%		N	%	N	%	
Age groups (years)										
16–19 years (n = 14)	12	85.7	2	14.3	$\chi^2 = 4.71$	9	64.3	5	35.7	$\chi^2 = 7.97$
20–30 years (n = 323)	274	84.8	22	6.8	P = 0.58	211	65.3	112	34.7	P = 0.048
31–40 years (n = 143)	126	88.1	6	4.2	(NS)	75	52.4	68	47.6	Significant
≥41 (n = 7)	6	85.7	0	.0		3	42.9	4	57.1	
Occupation										
Housewife (n = 400)	342	85.5	27	6.8	$\chi^2 = 7.69$	242	60.5	158	39.5	$\chi^2 = 5.25$
Teacher (n = 63)	55	87.3	3	4.8	P = 0.46	37	58.7	26	41.3	P = 0.26
Student (n = 12)	9	75	0	0.0	(NS)	10	83.3	2	16.7	(NS)
Nurse (n = 8)	8	100	0	0.0		7	87.5	1	12.5	
Governmental employee (n = 4)	4	100	0	0.0		2	50	2	50	
Obstetric History										
Gravida										
1 (n = 41)	124	87.9	8	5.7	$\chi^2 = 1.86$	95	67.4	46	32.6	$\chi^2 = 5.70$
2–4 (n = 201)	169	84.1	15	7.5	P = 0.76	125	62.2	76	37.8	P = 0.05
≥5 (n = 145)	125	86.2	7	4.8	(NS)	78	53.8	67	46.2	Significant
Parity										
0 (n = 166)	145	87.3	12	7.2	$\chi^2 = 4.4$	113	68.1	53	31.9	$\chi^2 = 7.99$
1 (n = 79)	68	86.1	5	6.3	P = 61	52	65.8	27	34.2	P = 0.046
2–4 (n = 177)	150	84.7	8	4.5	(NS)	97	54.8	80	45.2	Significant
≥5 (n = 65)	55	84.6	5	7.7		36	55.4	29	44.6	
Gestational age										
First trimester (n = 94)	80	85.1	8	8.5	$\chi^2 = 7.4$	56	59.6	38	40.4	$\chi^2 = 1.86$
Second trimester (n = 271)	233	86	11	4.1	P = 0.11	161	59.4	110	40.6	P = 0.39
Third trimester (n = 122)	105	86.1	11	9	(NS)	81	66.4	41	33.6	(NS)
Abnormal previous pregnancy outcomes										
Abortions										
0 (n = 325)	282	86.8	18	5.5	$\chi^2 = 2.24$	207	63.7	118	36.3	$\chi^2 = 3.32$
1–2 (n = 142)	120	84.5	11	7.7	P = 0.69	78	54.9	64	45.1	P = 0.19
≥3 (n = 20)	16	80	1	5	(NS)	13	65	7	35	(NS)
Malformed fetus										
Yes (n = 7)	7	100	0	0.0	$\chi^2 = 1.17$	2	28.6	5	71.4	$\chi^2 = 3.18$
No (n = 480)	411	85.6	30	6.3	P = 0.56	296	61.7	184	38.3	P = 0.074
					(NS)					(NS)

Table 4 Seroprevalence of *T. gondii* IgM and IgG antibodies in pregnant women by past history of infection and exposure to risk factor

Variable	<i>T. gondii</i> IgM				<i>P</i>	<i>T. gondii</i> IgG by ELISA					
	Negative		Positive			Negative		Positive		<i>P</i>	
	<i>N</i>	%	<i>N</i>	%		<i>N</i>	%	<i>N</i>	%		
Previous history of toxoplasmosis											
No (473)	406	85.8	29	6.1	$\chi^2 = 0.04$	293	61.9	180	38.1	$\chi^2 = 3.94$	
Yes (14)	12	85.7	1	7.1	$P = 0.98$ (NS)	5	35.7	9	64.3	$P = 0.047$ (Significant)	
Previous history of treatment with spiramycin											
No (475)	408	85.9	29	6.1	$\chi^2 = 10$	293	61.7	182	38.3	$\chi^2 = 1.97$	
Yes (12)	10	83.3	1	8.3	$P = 0.95$ (NS)	5	41.7	7	58.3	$P = 0.160$ (NS)	
Previous history of blood transfusion											
No (480)	412	85.8	30	6.3	$\chi^2 = 0.79$	295	61.5	185	38.5	$\chi^2 = 1.005$	
Yes (7)	6	85.7	0	0.0	$P = 0.67$ (NS)	3	42.9	4	57.1	$P = 0.316$ (NS)	
Consumption of undercooked meat											
No (483)	415	85.9	29	6	$\chi^2 = 2.7$	296	61.3	187	38.7	$\chi^2 = 0.213$	
Yes (4)	3	75	1	25	$P = 0.26$ (NS)	2	50	2	50	$P = 0.645$ (NS)	
Drinking unboiled goat milk											
No (477)	410	86	28	5.93	$\chi^2 = 4.0$	293	61.4	184	38.6	$\chi^2 = 0.54$	
Yes (10)	8	80	2	13.3	$P = 0.13$ (NS)	5	50	5	50	$P = 0.463$ (NS)	
Owing a cat											
No (472)	406	86	28	5.93	$\chi^2 = 1.71$	289	61.2	183	38.8	$\chi^2 = 0.14$	
Yes (15)	12	80	2	13.3	$P = 2.7$ (NS)	9	60	6	40	$P = 0.75$ (NS)	
Intake of immunosuppressive drugs											
No (<i>n</i> = 484)	417	86.2	29	6	$\chi^2 = 7$	296	61.2	188	38.8	$\chi^2 = 0.038$	
Yes (<i>n</i> = 3)	1	33.3	1	33.3	$P = 0.03$	2	66.7	1	33.3	$P = 0.845$	
Total	418	85.8	30	6.2	Significant					(NS)	

(Al-Mulhim and Al Qurashi 2001). Our results are also comparable to the results of a study done in Makkah, where the anti *Toxoplasma* IgG seropositivity was found to be 35.6 % (Ghazi et al. 2002). On the other hand, the results of the current study are somewhat higher than those of other similarly designed Saudi studies. In Makkah Al-Mukkaramah, the seroprevalence of *T. gondii* IgG & IgM were 29.4 % (58/197) and 5.6 % (11/197), respectively (Al-Harhi et al. 2006). In a recent Saudi study, anti *T. gondii* IgG & IgM seropositivity by ELISA were reported in 27.5 and 46.3 % of the studied pregnant women, respectively (Al Mohammad et al. 2010). In Jeddah, *T. gondii* IgG seropositivity was found in 25 % of the pregnant women (Tonkal 2008). On the contrary, the seroprevalence reported in this study was lower than that of a study done in Dammam area (42.1 %) (Abbas et al. 1986).

It was noticeable that the 38.8 % seroprevalence rate obtained in this study lies within the range of the average prevalence rate of *T. gondii* in Asian countries. High prevalence foci exist in the Middle East including Turkey, Iran, Iraq, Kuwait, Saudi Arabia, Jordan, Oman, and Lebanon (Pappas et al. 2009; Abu-Madi et al. 2008). In Palestine, the seroprevalence of *T. gondii* IgG among the studied pregnant women was 27.9 % (Nijem and Al-Amleh 2009). In Qatar, it was 35.1 % among women of the childbearing age (Abu-Madi et al. 2010). In Jordan, the seroprevalence of anti *T. gondii* IgM was reported to be 47.1 % among pregnant women (Jumaian 2005). As for Kuwait, the *T. gondii* IgG & IgM seroprevalence among pregnant women were reported to be 53.1 and 13.8 %, respectively (Iqbal and Khalid 2007).

This variation has been attributed to climate, cultural differences regarding hygienic and feeding habits, as well

as subpopulations' religious and socioeconomic practice (Pappas et al. 2009). The phenomenon of mixed racial populations may explain some of the observed differences which may be due to differences in the daily practices and ethnic habits (Chan et al. 2008). In Qatar, the increase in *T. gondii* IgG seropositivity was found among women coming from the East Mediterranean region or Africa than among others (Abu-Madi et al. 2008). Another important factor is the different methods used to determine the seroprevalence in the previously mentioned studies.

The high prevalence of *Toxoplasma* in this region (south western region) of the KSA could be attributed to that this region is observed to have a high population of cats (the final host of the parasite). Cats have been reported by Dubey (2009) as a high risk factor causes infection to the intermediate hosts including humans.

In the current study, we found no significant association between any of the women's characteristics and seropositivity for *T. gondii* IgM. Regarding exposure to possible risk factors, we found that pregnant women who reported history of intake of immunosuppressive drugs for different indications were significantly associated with *T. gondii* IgM seropositivity ($\chi^2 = 7$, $P = 0.3$). But exposure to various other risk factors was not significantly associated with *Toxoplasma* IgM seropositivity. These results are strongly supported by Petersen et al. (2010) who found that *T. gondii* cannot be explained by the presence of a risk factor.

In accordance with the results of the current study, a similar study on pregnant women in Makkah showed that the seroprevalence of IgM level had no significant difference among different age groups, whereas, the IgG level was significantly higher ($P < 0.05$) in older age groups. The highest level of *T. gondii* IgG seropositivity was found among pregnant women of 35–45 years (43 %). A recent Saudi study carried out in Al Hassa, also concluded that increased maternal age (>30 years) was significantly associated with increased risk of toxoplasmosis (Al Mohammad et al. 2010). The same finding was also reported in Qatar (Abu-Madi et al. 2008). The significant association with mother's age has been well known (Remington et al. 2001).

There was no significant association between *Toxoplasma* seropositivity (IgM or IgG) and the previous history of abnormal pregnancy outcomes as abortions or malformed babies. This is in agreement with the results of a previous Saudi study which denoted the absence of significant associations with previous history of abortions (Al-Harathi et al. 2006). In addition, another Saudi study reported that the perinatal outcome of pregnancy was remarkably good with no abortions, stillbirths, premature deliveries or congenital anomalies associated with *Toxoplasma* seropositivity (Al-Mulhim and Al Qurashi 2001).

In a recent study, fourteen case–control studies evaluating the importance of different risk factors for infection of humans with *T. gondii* were identified. Surprisingly, up to two-thirds of infections could not be explained by presence of risk factors (Petersen et al. 2010). In conclusion, considerable *T. gondii* IgM & IgG seropositivity by ELISA with limited identifiable significant risk factors are revealed in the study.

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