

Seroprevalence of *Toxoplasma gondii* in women in Najran City, Saudi Arabia

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ABSTRACT

Objectives: To evaluate the seroprevalence of *Toxoplasma gondii* among Saudi pregnant women in Najran City, as well as to measure the performance of the diagnostic tests used.

Methods: A total of 96 women attending prenatal special clinics (Oteafyn special clinic) in Najran Province, Saudi Arabia, over a one year period, from September 2012 to September 2013 were screened for the presence of *Toxoplasma* antibody in their blood serum using an indirect hemagglutination assay (IHA). Specific immunoglobulin (Ig) G and IgM antibodies were evaluated using an enzyme-linked immunosorbent assay (ELISA).

Results: Out of the 96 samples of sera tested using IHA, 20 (20.8%) were found to be positive with a titer ranging from 1:80 to 1:320, while 29 (29.2%) and 3 (3.1%) revealed *Toxoplasma* IgG and *Toxoplasma* IgM. A positive relationship was found between the seroprevalence of toxoplasmosis and age of tested women, especially in the age group of 21-30 years old (54.7%) by using ELISA-IgG, and 31-40 years old (4.5%) by using ELISA-IgM.

Conclusion: The seroprevalence of *Toxoplasma* among pregnant women was found to be comparatively high, compared with previous reports from Saudi Arabia. Serologic checkup before and during pregnancy for seronegative women is recommended.

Toxoplasmosis is an infection of homoeothermic vertebrates and one of the most common parasites of humans worldwide, infecting approximately one-third of the world's population. It is caused by the obligate intracellular protozoan parasite, *Toxoplasma gondii* (*T. gondii*). Its broad host range, high infection rate, worldwide distribution and the ability to maintain a benign coexistence with its host are features allowing it to be widely regarded as one of the most successful parasites on earth.¹

Toxoplasmosis gondii is a food-borne parasite, with cats playing the major role in its transmission, through

fecal contamination of soil, food, or water. Humans acquire infection by consumption of improperly cooked meat and unpasteurized goats' milk.² Detection of toxoplasmosis is currently dependent on serological techniques including screening for IgM and IgG antibodies, the former indicating recent infection while the latter indicating a past exposure and the existence of protective immunity.³ In Saudi Arabia, limited studies have been conducted to explore the seroprevalence of *T. gondii* among Saudi women. In previous studies the seroprevalence rates of toxoplasmosis by serological investigations have been estimated to be varied from 52.1% in Asir⁴ to 37.5% in Al-Hassa area,⁵ while the prevalence rate of anti-*Toxoplasma* IgG was 25%, and of IgM was 5% in the Eastern region.⁶

There is scarce information regarding *Toxoplasmosis* in the southern region of Saudi Arabia, particularly in the Najran area. Therefore, the objectives of this presented study are to explore the seroprevalence of *T. gondii* infection among pregnant women in Najran city, as a part of Saudi Arabia, to provide data for an educational program that will be designed to prevent *T. gondii* infection in women of childbearing age, and to determine the performance of serological tests used.

Methods. This study was conducted in the Southern region of Saudi Arabia, particularly in the Najran area, which lies between 17° 30' 20" North and 44° 11' 3" East. Almost 60% of the population lives in rural areas in close contact with livestock.

A single blood sample (5ml of blood by venipuncture) was taken from each participant (n=96) under aseptic conditions, all of which had been obtained from pregnant women within the age range of 20-40 years by using a questionnaire containing name, age and health history. Pregnant women with a known systemic disease (hypertension, diabetes mellitus, and so forth) were excluded. The women from different places (Alaresa, Al Khaledia and Alfyselia) in Najran city had been transferred for prenatal diagnoses at the Oteafyn special clinic (Department of Obstetrics and Gynecology) over a one year period from September 2012 to September 2013. Sera were separated and kept in sterile microtubes at -20°C until use for serological examinations.

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The collected sera were examined for detection of *T. gondii* antibodies using the indirect hemagglutination assay (IHAT) with a commercially available kit (TOXO-HAI, FUMOZUE Laboratories, Levallois-Perret, France) according to the manufacturer's instructions. In brief, sera were added to 96-well V bottomed polystyrene plates, and diluted in a 4-fold series from 1:80 to 1:2560. The plates were shaken for 2 minutes and then incubated at 37°C for 2 hours without shaking. The test was considered positive when a layer of agglutinated erythrocytes was formed in wells at dilutions of 1:80 or higher, and positive and negative controls were included in each test. Enzyme-linked immunosorbent assay (ELISA) was also used for the evaluation of anti-Toxoplasma IgG and IgM antibodies with a Vircell anti-*T. gondii* ELISA set (G1027 and M1027, Vircell S. L., Plaza Domínguez Ortiz, Granada, Spain). Antibody levels were evaluated by following the manufacturer's instructions at the laboratory of the Department of Applied Medical Sciences, Community College, Najran University, Najran, Saudi Arabia.

The significance of differences was analyzed using McNemar chi-square (χ^2) using the Statistical Package for Social Science version 15.0 (SPSS Inc., Chicago, IL), and $p < 0.05$ was considered significant. Additionally, the degree of agreement between the results from the 2 tests was quantified using kappa statistics and the accuracy

of the ELISA test in detecting exposure to *Toxoplasma* was evaluated in comparison to the IHA, and measured using the relative sensitivity and specificity.

The research proposal was approved by the Research Ethics Committee of the University and informed written consent was obtained from the subjects for blood sampling and information collecting.

Results. The obtained results showed that anti-*T. gondii* antibodies were found in 20 out of the 96 women (20.8%) with the IHA test. Eighteen samples were positive with a 1:80 titer and 2, with a 1:320 titer. In total, 31 (32.3%) samples of sera were found to be seropositive for toxoplasmosis by ELISA as displayed in Table 1. There was no significant difference detected between positive and negative results when comparing IHA and ELISA results with McNemar chi-square test ($p = 0.101$) and the strength of agreement between the 2 tests was considered to be good (Table 2).

All 96 samples were from the Najran area, 28 samples (29.2%), were seropositive for anti-*T. gondii* IgG, and only 3 (3.1%) were seropositive for anti-*T. gondii* IgM as determined by the ELISA test (Table 3).

The distribution of positive serum samples among different age groups for anti-*T. gondii* IgG showed that women in the age group of 21-30 years had the highest percentage (45.7%) of positive results followed by the age group 31-40 years (22.7%), while the age group >40 showed the lowest percentage (11.8%) (Table 3). This marked difference was found to be statistically significant ($\chi^2 = 8.436$, $p = 0.018$). The distribution of positive serum samples among different age groups for anti-*T. gondii* IgM showed that women of the age group 31-40 years had the highest percentage (4.5%) of positive results, followed by the age group 21-30 years (2.8%), while the age group >40 showed no infection. Although the age group of 31-40 years generally had a higher infection rate than other groups, it was not statistically significant ($\chi^2 = 0.85$, $p = 0.65$) (Table 3).

Table 1 - Indirect hemagglutination assay (IHA) and enzyme-linked immunosorbent assay (ELISA) seroprevalence of *Toxoplasmosis gondii* in women (n=96) from the Najran region, Southern Kingdom of Saudi Arabia.

Tests	Number of total samples	Number of positive samples (%)	Titer	
			1:80	1:320
IHA	96	20 (20.8)	18	2
ELISA	96	31 (32.3)	-	-
<i>P</i> -value = 0.101				

Table 2 - Diagnostic performance of indirect hemagglutination assay (IHA) and enzyme-linked immunosorbent assay IgG (ELISA IgG) of women (n=96) with antibodies against *Toxoplasma gondii*.

Variable	ELISA IgG		Sensitivity (Se)	Specificity (Sp)	κ value	SE of kappa
Serological tests	-Ve (negative) (n=68)	+Ve (positive) (n=28)	0.714 (0.511-0.860)	1 (0.933-1)	0.780 (0.637 - 0.922)	0.073
IHA (+Ve)	-0	20				
IHA (-Ve)	68	8				

Numbers in parentheses are 95% confidence interval, note-sensitivity=true positives detected/(true positives + false negatives); specificity=true negatives detected/(true negatives + false positives), SE - standard error

Table 3 - Seroprevalence and frequency of anti-*Toxoplasma gondii* IgG and IgM antibodies (ELISA Test) among different age groups of pregnant women from the Najran region, Southern Kingdom of Saudi Arabia.

Antibody types	Age range (years)	No. tested	No. positive (%)	No. negative (%)	OR (95% confidence)	95% CI	P-value
IgG	21-30	35	16 ^a (45.7)	19 (54.3)	8.906	1.765-44.948	0.018
	31-40	44	10 ^{b,c} (22.7)	34 (77.3)	2.06	0.430-11.322	
	> 40*	17	2 ^c (11.8)	15 (88.2)			
	Total	96	28 (29.2)	68 (70.8)			
IgM	21-30	35	1 (2.8)	34 (98.2)	1.522	0.059-39.352	0.65
	31-40	44	2 (4.5)	42 (95.5)	2.059	0.094-45.145	
	> 40*	17	0 (0)	17 (100)			
	Total	96	3 (3.1)	93 (69.9)			

^{a, b, c} value with different superscript in the same column differ at $p < 0.05$, *Reference category, OR - odds ratio, CI - confidence interval

Discussion. The current study was one of the few studies in Saudi Arabia, particularly the southern region, to explore the prevalence of *T. gondii* infection among one of the most important categories of people in whom toxoplasmosis occurs: women.

The seropositive rates of IHA were 20.8%, and of ELISA were 32.3%. This difference of positive rates may have resulted partly from the different antigenic epitope recognized by IHA and ELISA, and partly from a difference in the limits of sensitivity of both tests.³

According to the various studies conducted in Saudi Arabia, the seroprevalence varies from 25-52.1%. In our study, the overall seroprevalence among pregnant women was 32.3% by ELISA. It was concordant to some extent with a previous study (35.6%) from Makkah.⁷ Slightly lower rates were reported from healthy blood donors in 2 rural areas in the Eastern region of Saudi Arabia (25-26.36%),⁶ and a higher prevalence rate was reported in Asir region (52.1%).⁴ This fact underlined that *T. gondii* is a common protozoan in Southern Saudi Arabia.

Worldwide, similar reports were observed in Qatar as a seroprevalence of 35.1% was found among women of child-bearing age.⁸ However, other studies reported higher rates than our findings in some neighboring Arab countries like Jordan (37%).⁹ In contrast, the seroprevalence of *T. gondii* was higher as compared with other studies with a prevalence of 6.1% from northern Mexico.¹⁰ One of the reasons for this difference could be attributed to qualitative changes in hygiene and dietary habits, climate factors, socio-demographics as well as improvements in the quality of food preservation or changes in nutrition.

In the present investigation, the overall seropositivity to *T. gondii* among women for anti-IgG seroprevalence (chronic phase) was 29.2%, which means that around 70.8% of pregnant women were at risk of acquiring the infection if they were exposed during pregnancy, and consequently could transmit the infection to the fetus. Additionally, the anti-IgM seroprevalence (acute phase) was 3.1%, this reflects the risk among women with a recent infection who might transfer the parasite to the fetus.

Similar reports were described among pregnant women in Makkah, Saudi Arabia, as the percent of infection was 29.4% for anti-*Toxoplasma* IgG and 5.6% for anti-*Toxoplasma* IgM.¹¹ The 29.2% of *T. gondii* IgG positivity in pregnant women from Najran province was much lower than that in Brazil (49.2%).¹² On the contrary, 3.1% of *T. gondii* IgM positivity was slightly higher than Londrina, Paraná in Brazil (1.2%).¹²

Our results indicated the greatest risk of being infected with toxoplasmosis was in the age between 21-30 years as this age group comprised 45.7% of cases, followed by the 31-40 year age group (22.7%), while the least infected were in the age group >40 years (11.8%). These results corresponded with what was concluded previously in Duhok, Iraq as the majority of the participants were in their twenties (20-29 years), and constituted approximately 64.8% of cases.¹³ The results also are in concordance with those reported previously in Saudi Arabia, where 43% out of 86 pregnant women of the age group 21-30 years were more affected with toxoplasmosis.¹⁴ This also highlights the need to continue to educate women of childbearing age on the prevention of Toxoplasmosis.

In contrast, the present results were found to be different from most studies carried out in Saudi Arabia, as well as in the world that demonstrated a positive correlation between the seropositivity of *T. gondii* IgG and older ages.^{3,13} This data suggest that transmission of *T. gondii* in the general population has been stable in some groups and localities over the past 3 decades, whereas in others it appears to be increasing. Transmission may have mainly occurred early in life (congenital or childhood). However, transmission appears to continue during adulthood.

The present data showed that the highest level of seroprevalence of IgM was among the 31-40 year age group (3.1% IgM seropositivity); a result that is in disagreement with the published findings that show the most frequent seroprevalence in the 15-35 years age groups.¹⁵ Toxoplasmosis remains a problem, mainly in risk groups such as pregnant women and immunocompromised patients. Improvement can only be attained by increasing prevention and reducing risk factors. It is essential to know the distribution and seroprevalence of *T. gondii* infection among pregnant women. The results showed that women in Najran state are susceptible to the toxoplasmosis parasite. Thus, the implementation of regular serological testing during pregnancy is important to reduce the effects of the disease on mothers as well as on newborn babies.

In conclusion, this survey is important because it can be considered as a baseline for further studies. Furthermore, the current investigation showed that seroprevalence of Toxoplasmosis among pregnant women was found to be comparatively high (PubMed search), compared with previous reports from Saudi Arabia with a higher anti *T. gondii* IgG seropositivity among participants aged 21-30 years old. Further focused studies should be conducted; taking into consideration other possible infection risk factors that were not considered in this study, so to determine the common sources of Toxoplasma transmission.

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