Seroprevalence of *Toxoplasma gondii* among pregnant Saudi woman in Arar, Northern Borders Province, Saudi Arabia

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Background
Toxoplasmosis, a worldwide disease, is considered one of the main problems challenging developed and developing countries.
Congenital toxoplasmosis is caused by intrauterine infection with Toxoplasma gondii. Particularly, if the infection occurs in the first trimester of pregnancy it may cause serious complications, resulting from vertical transmission to an embryo, such as microcephaly, hydrocephalus, and blindness. This study aimed to investigate the seroprevalence of T. gondii among pregnant Saudi women in Arar, Northern Borders Province, Saudi Arabia.
Patients and methods
A total of 340 participants enrolled for prenatal care at the Arar Maternity and Pediatric Hospital in Arar over a 1-year period between January 2015 and January 2016 were included. Two techniques were used to detect the presence of *T. gondii*-specific antibodies in their sera: an indirect hemagglutination assay followed by a specific enzyme-linked immunosorbent assay.
Results
In general, the data showed that there was a positive correlation ($P<0.05$) between women aged between 20 and 30 years and toxoplasmosis infection.
Of the 340 samples tested using an indirect hemagglutination assay, 285 samples were negative and 55 (6.2%) samples were positive at dilutions between 1 : 64 and 1 : 2048.
For the specific enzyme-linked immunosorbent assay, from 340 tested samples, two were seropositive at 0.6% with *T. gondii* immunoglobulin M and 55 samples were seropositive at 13.5% with anti-*T. gondii* immunoglobulin G.
Conclusion
Screening measures can be taken to decrease the risk for infection during pregnancy and prevent severe illness in newborn infants. Therefore, many cases of congenital toxoplasmosis can be prevented.

Keywords:
Arar city, ELISA, pregnant women, Saudi Arabia, seroprevalence, *Toxoplasma gondii*

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Introduction

Toxoplasma gondii, an obligate intracellular parasite, is known as the causative agent of toxoplasmosis infection that can infect humans and various types of animals [1]. It is highly infectious and widespread [2]. *T. gondii* is a common public health problem, being present in both developed and developing countries [3].

T. gondii infection can be asymptomatic to mild in healthy individuals [1]. Spontaneous abortion and stillbirth as well as neonatal morbidity and mortality have been linked with congenital toxoplasmosis, particularly when it occurs in the first trimester of pregnancy [3]. Its vertical transmission to an embryo can cause serious complications such as microcephaly, hydrocephalus, blindness, and fetal death [4–6].

The main pathways for *T. gondii* transmission are thought to be the ingestion of food and water contaminated by feline fecal matter, soil contaminated with oocysts, or tissue cysts in raw and undercooked meat [7].

The number of babies affected by congenital infection of *T. gondii* in the USA is estimated to be one in every 10 000 normal births [8]. The frequency is higher in Mexico with two cases per 1000 normal births [9].

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In Saudi Arabia, several studies have been conducted to investigate the seroprevalence rates of toxoplasmosis among pregnant Saudi women (PSW), but no study has been undertaken in the Northern Boarders Province of Saudi Arabia. The aim of the present study was to estimate the prevalence of *T. gondii* among PSW in Arar.

Patients and methods

Study area and population

Blood samples were obtained from 340 participants including five positive and five negative controls. The study is based on women who were referred to prenatal care at Arar Maternity and Pediatric Hospital and their ages ranged between 20 and 40 years.

The participants were divided into four age groups: 20–25, 26–30, 31–35, and 36–40 years old.

The current study covered most areas located within Arar (30°59′00″N 41°01′00″E) between January 2015 and January 2016. The purpose and procedure of the study were explained to the patients and they signed a consent form agreeing to be involved in the study.

Sampling

Blood samples (5 ml of blood without anticoagulant in a vacutainer; BD company, Franklin Lakes, New Jersey, United States) were collected under aseptic condition using venepuncture of a radial vein from the PSW who had been referred to prenatal care at Arar Maternity and Pediatric Hospital. The clotted blood samples were then centrifuged at 2000 rpm/min for 10 min in order to separate the serum, which was then transferred into separate tubes and stored at −20°C until analysis.

Detection of *T. gondii* (immunoglobulin G) using the indirect hemagglutination assay technique

The separated serum, from the collected samples in this study, was tested using a commercially available indirect hemagglutination assay (IHA) test kit (Cellognost Toxoplasmosis H; Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). The IHA test was performed in accordance with the manufacturer's instructions using a polystyrene V-bottomed microtitration plate (96-well).

The patients' sera, with the positive and negative controls provided with the kit, were added to the plate after dilution with the kit's serum buffer or 0.2 mol/l amino-2-methyl-1-propanol (25 µl of participant's serum in 175 µl of serum buffer). Serial dilutions (from 1 : 8 to 1 : 32768) were applied to the other wells, which contained 50 µl serum buffer. Thereafter, 50 µl of toxoplasmosis H IHA reagent was added to all dilutions. The plate was then shaken and incubated for 2–24 h at 37°C.

The tests were interpreted as follows: the antibody titers were identified by the last dilution of erythrocyte agglutination that participated in the middle last of dispersion. The serum was considered positive according to the manufacturer's recommendation at dilutions of 1 : 16 or higher (up to 1 : 32768).

Detection of *T. gondii* immunoglobulin M and immunoglobulin G antibodies using the enzyme-linked immunosorbent assay technique

The participant's sera were tested to detect the specific antibodies [antitoxoplasma immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies] of *T. gondii* using the enzyme-linked immunosorbent assay (ELISA) technique (United Diagnostics Industry). Toxoplasma ELISA set was used (ref no. EM127 IgM and EG127 IgG; Dammam, KSA).

Both IgM and IgG tests were performed according to the manufacturer's instructions. The results of the tests were obtained in optical density readings at 450 nm, using a virology analyzer machine (EVOLIS; Bio-Rad). The ratio was calculated and compared with the optical density value of the sample and the cutoff. For both IgM and IgG, the sample was considered positive if the ratio was more than 1.100, negative if the ratio was less than 0.900, and doubtful if the ratio was 10% more or less of the cutoff (0.90–1.10) according to the manufacturer's instructions.

For the ELISA technique, the first well is considered as a blank. To initialize the procedure, 100 µl of negative, calibrator, positive control, and sample were added into the selected well plates. The plates were then incubated for 45 min at room temperature. After the incubation period, the plate content was aspirated and washed four times with washing solution. After the first washing cycle, 100 µl of conjugate was added into each well and incubated at 37°C for 45 min. A second washing cycle was repeated four times, for 30 s each time, with washing solution. At the end of the second wash, 100 µl of tetramethyl blue chromogen solution was added into each subsequent well and incubated for 15 min at room temperature. A 100 µl of stopping solution was added after the last incubation. Finally,

the absorption of the solution in the wells was read within 30 min at 450 nm according to the manufacturer's instructions.

Statistical analysis

The study data were analyzed using the χ^2-test and *t*-test using IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, New Your, USA) to investigate the significant differences between the two test groups. A *P*-value is considered a significant result if *P* is less than 0.05. Furthermore, relative specificity and sensitivity were used to determine the accuracy of the diagnostic tests used in this study.

Results

Detection of *T. gondii*-specific-antibodies (immunoglobulin G) using the indirect hemagglutination assay technique

Sera from 340 participants were tested to investigate the seroprevalence of the *T. gondii* antibody titer using the IHA technique.

A large number of the samples, 285 of 340, were negative; however, a small proportion of samples, 35 out of 340, were positive at different dilutions ranging between 1 : 64 and 1 : 2048 (Table 1). The IHA titer results showed one (1.8%) case with the *T. gondii* antibody titer of 1 : 64, 34 (61.8%) cases with 1 : 128, seven (12.7%) cases with 1 : 256, 10 (18.2%) cases with 1 : 512, two (3.6%) cases with 1 : 1024, and one (1.8%) case with 1 : 2048. In total, the percentage of IHA-positive tests from 344 samples was nearly 16.2% and the percentage of negative IHA tests, out of the total proportion, was about 83.8% (Table 2).

Detection of *T. gondii*-specific antibodies (immunoglobulin M and immunoglobulin G) using the enzyme-linked immunosorbent assay technique

In addition to the IHA technique, toxoplasma IgM and IgG ELISA assays were completed in order to increase the accuracy of the diagnoses and identify recent infection.

According to the seropositivity of the tested samples, the results showed two samples of antitoxoplasma IgM and 46 samples of antitoxoplasma IgG as seropositive as determined using the ELISA technique at 0.6 and 13.5%, respectively (Table 3).

Comparison between the IHA and the toxoplasma IgM and IgG antibodies showed that the results were significant ($P<0.05$). In addition, comparison between IHA and both ELISA assays showed a sensitivity of 100% and a specificity of 96.9% for the IHA test (Table 2). The strength of agreement between these two assays was also considered as significant ($P<0.05$) (Table 4).

Characteristics of *T. gondii* seropositive patients

The participants' age distribution of the samples positive for toxoplasma antibodies is shown in Table 5, and the titers of the IgG antibody of the serum samples measured using ELISA are shown in Fig. 1. On the basis of the IHA test, the percentage of positive samples was 16.2%. However, the percentages of ELISA-positive tests

Table 1 Distribution of indirect hemagglutination assay titers with their relative frequency, percent, and standard error

IHA titers (dilutions)	Frequency	Valid (%)	Standard error	95% confidence interval	
				Lower	Upper
1 : 64	1	1.8	1.9	0	7.3
1 : 128	34	61.8	7.1	47.3	74.5
1 : 256	7	12.7	4.8	5.5	23.6
1 : 512	10	18.2	5.3	7.3	29.1
1 : 1024	2	3.6	2.7	0	9.9
1 : 2048	1	1.8	1.7	0	5.5
Total	55	100	0	100	100

All the indirect hemagglutination assay (IHA) dilutions in this table were graded as positive titration.

Table 2 Comparison between indirect hemagglutination assay and enzyme-linked immunosorbent assay assays in relation to their seropositivity of *Toxoplasma gondii* in 340 samples

	ELISA (IgM and IgG)		Total
	Positive	Negative	
IHA			
Positive			
Count	46	9	55
%Within ELISA (IgM and IgG)	100.0	3.1	16.2
Negative			
Count	0	285	285
%Within ELISA (IgM and IgG)	0.0	96.9	83.8
Total			
Count	46	294	340
%Within ELISA (IgM and IgG)	100.0	100.0	100.0

When compared with the indirect hemagglutination assay (IHA), the enzyme-linked immunosorbent assay (ELISA) [immunoglobulin M (IgM) and immunoglobulin G (IgG)] tests had a sensitivity of 100% and specificity of 96.9%. $P<0.05$.

Table 3 Frequency distribution of anti-*Toxoplasma gondii* immunoglobulin M and immunoglobulin G with their relative percentage

	ELISA IgM [*n* (%)]		ELISA IgG [*n* (%)]	
	Positive	Negative	Positive	Negative
Count	2 (0.6)	338 (99.4)	46 (13.5)	294 (86.5)
Total	340 (100)	340 (100)		

ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IgM, immunoglobulin M.

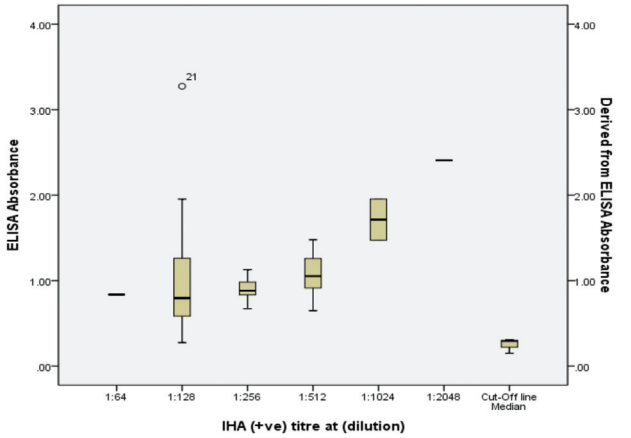
Table 4 Measuring of indirect hemagglutination assay and enzyme-linked immunosorbent assay agreement using Cohen's κ coefficient

	Value	Asymptomatic standard error	Approximate *T*	Approximate significance
Measure of agreement (κ)	0.895	0.034	16.603	0.000
Number of valid cases	340			

Table 5 Frequency of the age distribution based on the positive indirect hemagglutination assay and enzyme-linked immunosorbent assay (immunoglobulin M and immunoglobulin G) assays

Age (years)	IHA [*n* (%)]		Total [*n* (%)]	ELISA (IgM and IgG) [*n* (%)]		Total [*n* (%)]
	Positive	Negative		Positive	Negative	
20–25	25 (7.4)	158 (46.5)	183 (53.8)	22 (6.5)	161 (47.4)	183 (53.8)
26–30	23 (6.8)	102 (30.0)	125 (36.8)	18 (5.3)	107 (31.5)	125 (36.8)
31–35	7 (2.1)	24 (7.1)	31 (9.1)	6 (1.8)	25 (7.4)	31 (9.1)
36–40	0 (0.0)	1 (0.3)	1 (0.3)	0 (0.0)	1 (0.3)	1 (0.3)
Total	55 (16.2)	285 (83.8)	340 (100.0)	46 (13.5)	294 (86.5)	340 (100.0)

Figure 1



Seropositive incidents of *T. gondii* infection obtained by ELISA and it's correlation with IHA techniques. The ELISA absorbance cut-off was 0.295

were 0.6 and 13.5% for IgM and IgG, respectively (Table 3).

There is a decrease in the prevalence of toxoplasma antibodies with increasing age. This decrease was statistically significant ($P>0.05$): 20–25 years old (7.4%); 26–30 years old (6.8%); 31–35 years old (2.1%); and 36–40 years old (0%).

Discussion

Understanding the prevalence of *T. gondii* infection in the general public is important. This study aimed to estimate the seroprevalence of *T. gondii* infection rate among PSW in Arar.

The prevalence of toxoplasmosis has been linked to several factors such as different climates in different regions and rural or urban settings [10]. Several other studies [2,11,12] reported different rates of *T. gondii* seroprevalence across Saudi Arabia – 52.1% in Asir, 37.5% in Al-Hassa, 25% in eastern regions, and 54.7% in Najran. The rate in our study was 13.5%, which is much lower than those reported in other Saudi Arabia studies.

In countries neighboring to Saudi Arabia, such as Qatar and Iraq, the overall rate was found to be 29.8 and 20.1%, respectively [1,13]. Furthermore, a much higher rate of 37% was reported in Jordan. However, a lower rate of 6.1% was reported in northern Mexico [14]. These significant differences between studies and/or countries may be attributed to climate change [3], nutritional behavior, location of residence, food preservation, cat abundance [1], study population, sample size, age, sensitivity of serological techniques used, *T. gondii* oocyst survival in the environment [15,16], and family medical practitioners who have lacked relevant education about *T. gondii* transmission [17].

IHA (16.2%) and ELISA (13.5%) tests showed differences related to the different antigenic determinant and/or partly from different sensitivity limits for each test [2].

In our results, the seroprevalence of *T. gondii* decreased with increasing age. This result differs from other results from Nigeria and Congo [18,19]. The age group in our study at greatest risk for *T. gondii* infection comprised those between 20 and 25 years at a rate of 6.5%, with 22 cases of 46 being ELISA positive. The second highest seroprevalence rate was observed in the group aged between 26 and 30 years at 5.3%. These results correlate with results from Ra'ad ADdory and colleagues [20–22], who reported more positive cases in their study group aged between 20 and 29 years than other ages.

These findings were also reported in Al-Hindi and Lubbad [23], which reported that the age of PSW infected by *T. gondii* was between 21 and 30 years. Moreover, a study conducted in Gaza, Palestine [24] reported that the age group between 23 and 28 years is more exposed and at risk for *T. gondii* infection.

A greater exposure to pets may explain a higher infection rate [25,26]. For example, a low level of hand sanitization before and during human food preparation combined with cats' poor sanitary habits and a low awareness of toxoplasmosis could lead to higher rates of infection.

However, Osiyemi *et al*. [17] have suggested that health counseling for pregnant women about the risk factors for *T. gondii* infection may reduce the incidence of congenital toxoplasmosis. Other studies [27,28] that focused on counseling pregnant women about the risk factors for *T. gondii* infection have proven to be effective and successfully decreased the incidence of congenital toxoplasmosis.

In conclusion, this study has established the seroprevalence of *T. gondii* among PSW in Arar. The overall prevalence of *T. gondii* is lower in the study area (Arar) than in other areas of Saudi Arabia. It was noticed that there is a reverse relationship between the seroprevalence of *T. gondii* and the age of PSW; the prevalence decreases with increasing age.

The highest rate of seroprevalence of *T. gondii* among PSW was among those between 20 and 25 years of age. Educating pregnant women is necessary for increasing the level of awareness on the transmission mode of *T. gondii* and prevention.

Further investigation, including the risk factors and the transmission mode of *T. gondii*, is recommended.

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Conflicts of interest
There are no conflicts of interest.

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