Seroprevalence and Risk Factors for Toxoplasmosis in Pregnant Yemeni Women

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Abstract

Objectives: The current study conducted to determine the profile of toxoplasmosis in pregnant Yemeni women, and to identify potential risk factors that could be associated with infection. Methods: Using a cross-sectional approach, a total of 481 pregnant women, aged ≤ 20 to >36 years, attending the general hospitals in three cities named Emran, Taiz, and Sana'a were studied, during the period from February to September 2012. Relevant data were collected from each participant by means of questionnaire, and serum samples screened for anti-toxoplasma antibodies, IgG and IgM by enzyme-linked immunosorbent assay (ELISA). The correlation between positivity rate of IgG Abs, and some socio-demographic risk factors was assessed. Results: Out of the 481 women investigated, 176 (36.6%) and 10 (2.1%) were positive for anti T. gondii IgG and IgM respectively. Age, educational level, and drinking unpasteurized goat's milk were the risk factors that significantly associated with a higher rate of IgG Abs. Cats contact, handling raw meat, and consumption of undercooked meat as well elevated the rate of IgG Abs in spite of being not statistically significant. Conclusion: It can be concluded from this study that, the seropositivity rate of IgG Abs to T. gondii is a substantial, and nearly similar to the rates stated from other countries in Arabian Peninsula. Age, educational level, and drinking unpasteurized goat's milk were the risk factors that significantly associated with a higher rate of IgG Abs.

Key words: risk factors, toxoplasmosis, pregnant, Yemeni women, ELISA

INTRODUCTION:

Toxoplasmosis is a cosmopolitan disease, arising from infection with the cat-borne coccidian protozoan, Toxoplasma gondii. It is an obligate intracellular organism that forms cysts in mammalian tissues throughout the body.1 Approximately one third of the world's human populations is infected by such protozoan.2 The parasite infects most genera of warm-blooded animals, including humans. It rarely causes any symptoms in otherwise healthy adults. However, among those with a weakened immune system, as AIDS patients, it may cause fatal disease.3 The infection is acquired by ingestion of mature oocysts in water and / or food contaminated with cat feces, and by eating raw or undercooked animal's meat containing infective tissue cysts. Infection may also happen congenitally from pregnant acquired primary infection to her fetus through transplacental transmission.4-6 Maternal toxoplasmosis is usually asymptomatic; but, if the diagnosis is delayed, irreversible fetal infection may take place, leading to spontaneous abortion, still birth; or serious fetal damage such as, encephalitis, hydrocephalus, and chorioretinitis.7

Serological testing is the mainstay for the diagnosis of toxoplasmosis. The analysis is based on the positivity of anti T. gondii specific immunoglobulin G (IgG),"evidence of earlier infection, peaking at 4 months after infection and persisting at low levels for life"; and on anti T. gondii specific immunoglobulin M (IgM),"evidence of current or

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recent infection, appearing within 1–2 weeks of infection and subsiding by 6–9 months".8

In Yemen, seroprevalence studies are little concerned with Toxoplasma infection among pregnant women, as well the lacking reports of its risk factors. Description of the epidemiology of toxoplasmosis may recognize socio-demographic risk factors that may contribute to the disease. The objective of the present study was to establish the seroprevalence of Toxoplasma gondii infection in pregnant Yemeni women and to identify potential risk factors that could be associated with infection.

SUBJECTS AND METHODS

Study subjects and sampling

This cross- sectional approach was carried out during the period from February to September 2012. A total of 481 pregnant women, aged ≤ 20 to >36 year were selected. They were attended the antenatal clinics of general hospitals from three cities; 109 samples were collected from Emran General Hospital(inside Emran city), 175 from Al-Gomhoree Hospital (Taiz city), and 197 from Al-Sabeen Hospital (Sana'a city). Medical sheets were filled out on each participant included; age, education level, contact with cats, handling raw meat, consumption of raw or undercooked meat, and consumption of raw or unpasteurized milk. Using a sterile disposable syringe, 5ml of venous blood sample were collected from every subject. Samples were centrifuged, then the sera separated. Samples were transferred into Eppendorff tubes, and stored at -20°C until being processed. Each sample was then checked for anti-T. gondii IgG and IgM using commercially available ELISA.

Immunoenzymatic assay (PlateliaTm)

An enzyme-linked immunosorbent assay was performed in vitro using PlateliaTmBio-Rad; France kit for the detection the anti-Toxoplasma gondii antibodies in women sera, according to the manufacturer′s instructions. The assay was of two types. The first is an indirect ELISA (solid phase immunoassay), PlateliaTmToxoIgG; 72840, that was applied for the detection and titration of IgG

antibodies. Briefly, serum samples and controls were diluted 1/21, and distributed in the wells of microplate. During incubation for one hour at 37oC, IgG antibodies(Abs) to T.gondii present in the sample bind to T. gondii antigen(Ag), coated on microplate wells. After incubation, unbound non specific Abs and other serum proteins are removed by washings. The conjugate (peroxidase labeled monoclonal Ab specific for human gamma chains) is added to the microplate wells. During incubation of one hour at 370C, labeled Ab binds to serum IgG captured by T.gondii Ag. The unbound conjugate is removed by washings at the end of the incubation. The presence of immune complexes is demonstrated by addition in each well of an enzymatic solution. After incubation in the dark for a half hour at room temperature, the enzymatic reaction is stopped by addition of 1N sulfuric acid solution. The optical density reading obtained with a spectrophotometer set at 450/620nm is proportional to the amount of IgG Abs to T.gondii present in the sample. The second system is an indirect ELISA(with capture of the IgM on the solid phase immunoassay), PlateliaTmToxoIgM; 72841. The assay was conducted as for IgG except that; antibody to human IgM was used to detect IgM Abs to T.gondii in human serum bound to the plates.

DATA ANALYSIS:

The data in questionnaires were transcribed onto computer coding sheets, and statistically analyzed by Epi-info version, 2000 (CDC, Atlanta, GA, USA).The results were tested by Chi-square (X2) test for significance between categorical variables. A value of P<0.05 was considered………significant.

Ethical consideration: Ethical approval was yielded from the hospitals. The purpose of research was explained to the participating women, and informed consent was obtained from each.

RESULTS

Out of 481 pregnant women examined, 176 (36.6%), and 10 (2.1%) were positive for anti T.gondii IgG and IgM

respectively (Table 1). The percentages positivity of IgG Abs in the cities, Emran, Taiz and Sana'a were nearly similar, 38.5%, 34.9%& 37.1% respectively (Table 2). From table 3, the positivity rate of IgG Abs increased significantly as the pregnant's age increased. Therefore, those aged $\leq$ 20 and 21-28 year represented the lowest positivity rates (34.4%& 32.4% respectively); whereas, those aged >36 year represented the highest one (72.4%); (X2 = 17.98, P = 0.000). It is evident from the table that, the positivity rate of IgG Abs decreased significantly as the subject's educational level increased; (X2 = 40.96, P<0.000). Moreover, drinking unpasteurized goat's milk was recognized as a further significant factor for toxoplasmosis among pregnant women; (X2= 33.79, P <0.000). Another factors including, cats contact, handling raw meat, and consumption of undercooked meat also elevated the rate of IgG Abs despite being not statistically significant.

Table 1: Seroprevalence of anti T. gondii IgG and IgM in pregnant women (ELISA)

Antibody	No. examined	No. +ve	%
IgG	481	176	36.6
IgM	481	10	2.1

Table 2: Seroprevalence of anti T. gondii IgG among pregnant women in the studied cities

City	No. examined	No. +ve	%
Emran	109	42	38.5
Taiz	175	61	34.9
Sana'a	197	73	37.1
Total	481	176	36.6

Table 3: Seroprevalence of anti T. gondii IgG among pregnant women according to socio-demographic characteristics

Character	No. examined	Positive		Negative		X² (P value)
		No.	%	No.	%	
Age groups(in years)						
$\leq$ 20	128	44	34.4	84	65.6	
21-28	210	68	32.4	142	67.68	**17.98 (0.000)**
29-36	114	43	37.7	71	62.3	
> 36	29	21	72.4	8	27.6	
Education						
Secondary or university	107	16	14.9 20.6	91	85.1	**40.96 (<0.000)**
Primary or preparatory	151	48	31.8	103	68.2	
Illiterate or read and write	223	112	50.2	111	49.8	
Contact with cats						
No	397	145	36.5	252	63.5	**0.00 (0.947)**
Yes	84	31	36.9	53	63.1	
Handling						
No	73	25	34.2	48	65.8	**0.20 (> 0.652)**
Yes	408	151	37.0	257	63.0	
Meat						
Sufficiently cooked	425	152	35.8	273	64.2	**1.07 (< 0.300)**
Undercooked	56	24	42.9	32	57.1	
Drinking						
No	112	15	13.4	97	86.6	**33.79 (<0.000)**
Yes	369	161	43.6	208	56.4	
Total	481	176	36.6	305	63.4	

DISCUSSION

Toxoplasma gondii is a widely prevalent parasite that is potentially responsible for significant morbidity and mortality in the congenitally infected children and those with immunosuppression and for high morbidity in all persons in the form of ocular disease.9 -It is one of TORCH infections group "Toxoplasma gondii, Rubella, Cytomegalo-virus and Herpes simplex virus".10 If a

woman gets infected with T. gondii for the first time in her life during pregnancy, she may pass infection to her fetus; a situation that ultimately could lead to a very serious fetal damage.7

In the present study, the rate of IgG Abs to T. gondii was found to be more common than rate of IgM Abs. It was 36.6%. Nearly similar rates, 35.6%, and 35.1% were reported from Makkah, Saudia Arabia; and Doha, Qatar respectively.5,11 Lower seroprevalence rates were stated from United Arab Emirates, Palestine, and Turkey, 22.9%, 27.9%, and 30.1% respectively. 12-14 In contrast, higher rates of 58.2% and 57.0% were recorded from Kuwait and Egypt respectively.15,16 Differences in the incidence of Toxoplasma infections from one country to another or even within the same country may be attributable to the impact of climatic, cultural variations regarding hygienic and feeding habits.9 The percentages positivity of IgG Abs were nearly similar in the three studied cities. In the current study, the rate of IgG Abs among pregnant women increased significantly as the age increased. Thus, those aged $\leq$ 20, and 21-28 year represented the lowest rates of infection; whereas, those aged >36 year represented the highest one (X2 = 17.98, P = 0.000). It was deduced that, the rate of infection increased gradually as the age increased and this relation proves the fact that, the greater the prevalence, the earlier the rise. This association might be explained by, the older the individual, the longer time being exposed to the causative agent and may retain a stable level of IgG Abs in serum for years.10,17 .

The positivity rate of immunoglobulin G among the studied subjects, decreased significantly as the educational level increased(X2 = 40.96, P<0.000). Higher educational level may limit the exposure toward infection and increases consciousness to undertake the sanitary modes concerning habits of cooking and food, that may lead to reduce in toxoplasmosis.17

Drinking unpasteurized goat's milk was identified as a further significant risk factor for toxoplasmosis in the

present findings(X2= 33.79, P <0.000). Unpasteurized goat's milk is thought to be contaminated by T. gondii oocysts that originate from cat feces. The current result goes in accordance with other study that carried among American case patients where, drinking unpasteurized goat's milk elevated the risk of T. gondii infection.9 In contrast, consumption of raw milk by Iranian pregnant women was not statistically associated with T. gondii infection.17 This discrepancy may be ascribed to the effect of lifestyle and practices with regard to feeding customs.   In results of the current work, statistically insignificant association between the positivity of IgG Abs and other factors, does not indicate the ineffectiveness of these factors on the spread of toxoplasma. In other study, such factors were reported to elevate the risk of toxoplasma infection.18 This variation may be due to the impact of educational behaviors and attitudes of subjects in the present study; as well as, the effect of climatic factors. Stray cats are common in Yemen and generally, they are regard as the major source of toxoplasma infection passing the oocysts; though, the hot and dry climate conditions are not ideal for oocysts survival, compared to cooler, more humid and rainy environment.19 The majority of Yemeni people do not usually consume raw or undercooked meat in the diet; but, the consumption of raw or unpasteurized milk is frequent by them.

The overall seropositivity rate of IgM Abs was at a low level in the present work(2.1%). It has been stated that, the seropositivity of anti T. gondii IgM suggesting current or recent infection. IgM Abs develop at the beginning, their titres persist for weeks and subside within few months of infection.8 However, in other surveys, the positivity rates of IgM Abs are stated in the chronic stage of infection and may continue after the acute of infection. For this rationalization, the sero-positivity of IgM Abs is not essentially an evidence of current infection.10,20,21

CONCLUSION

It can be concluded that, the seropositivity rate of IgG

Abs to T. gondii in this study is a substantial, and nearly similar to the rates stated from other countries in Arabian Peninsula. Age, educational level, and drinking unpasteurized goat's milk were the risk factors that significantly associated with a higher rate of IgG Abs. Further studies are ongoing with the role of drinking water source, and polluted vegetables as the possible risk factors for toxoplasmosis notably in the rural areas. Moreover, the association between toxoplasmosis and the occurrence of abortions among pregnant women should be studied. Health education programs should be designed for pregnant women to prevent primary infection, and must concentrate on the effect of behavioral practices on such disease.

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