

Sero-prevalence of toxoplasmosis among pregnant women attending an ante-natal clinic at a teaching hospital in Al Kharj, Saudi Arabia

Qamer, S.^{1*}, Rizvi, S.S.R.², Raoof, S.³, Kamal, S.M.⁴ and Khan, S.⁵

¹Department of Basic Medical Sciences, Division of Microbiology, College of Medicine, Prince Sattam bin AbdulAziz University, Alkharj, 11942, Saudi Arabia

²Department of Pathology, Frontier Medical & Dental College, Abbottabad, Pakistan

³Department of Obstetrics and Gynecology, Sindh Government Hospital New Karachi, Pakistan

⁴Department of Medicine and Infectious disease, Prince Sattam Bin Abdul Aziz University, Alkharj 11942, Saudi Arabia

⁵Department of Biochemistry, College of Medicine, Al Imam Mohammed Ibn Saud Islamic University, Riyadh, Saudi Arabia

*Corresponding author e-mail: drqamar98@gmail.com

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Abstract. *Toxoplasma gondii* (*T. gondii*) is a zoonotic infection that may be transmitted to human beings either by consumption of raw or uncooked meat or by ingesting oocysts. *Toxoplasma* organisms can cross blood placenta barrier and may result in congenital toxoplasmosis. About 80% of immunocompetent individuals do not show any clinical manifestations and are silent carriers of this disease. Pregnant women especially in highly prevalent areas are recommended to be screened for this disease in order to prevent the potential vertical transmission. To our knowledge no such study has been conducted in this region of Saudi Arabia. This study attempted to carry out two objectives: first, to find out the seroprevalence of *T. gondii* infection in pregnant women attending prenatal care services in our hospital; second, to find out risk factors associated with *T. gondii* seroprevalence in our patients. It was carried out in Teaching Hospital in Al-Kharj over a period of one year. All 306 pregnant women attending antenatal clinic were involved in the study. A pretested self-explanatory questionnaire was filled out by the patients and their sera were collected to be tested for IgG and/or IgM against *T. gondii*. The results were then statistically analyzed using SPSS software and p-value was calculated using Pearson Chi Square test. Out of the 306 blood samples tested, 99 (32.4%) were seropositive for specific anti *T. gondii* IgG antibodies and 3(1%) were seropositive for IgM. This show that seroprevalence of *T. gondii* antibodies was high among pregnant women and the prevalence showed a significant association with age. The study recommends conducting educational programs to raise awareness among women about risk factors and precautions to be taken.

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is an obligate intracellular parasite that primarily infects numerous animals including human beings (Alzaheb & Al-Amer, 2017). It is transmitted from animals to humans by either of the following three routes: (a) Consumption of raw or undercooked meat contaminated with cysts of parasites (Jones *et al.*, 2006);

(b) ingesting oocyst through contact with infected cat feces or by touching contaminated soil or consuming food or water contaminated with oocyst (Joyner, 1982); (c) Vertical transmission from infected mother to fetus through placenta (Rorman, Zamir, Rilkis & Ben-David, 2006). This zoonotic infection can result in toxoplasmosis in humans which is a global disease (Alzaheb & Al-Amer, 2017).

Toxoplasmosis can be lethal in high risk groups like pregnant women and immunocompromised individuals. Global prevalence of toxoplasmosis is 30-50% and Middle East has the highest prevalence (Alzaheb, 2018; Ahmadpour, Daryani & Sharif, 2014; Pappas, Roussus & Falagas, 2009).

Almost 80% of the immunocompetent individuals infected with *T. gondii* have no clinical manifestations. Although oocyst of *T. gondii* can be isolated from CSF, uteroplacental circulation, etc. via immunofluorescent technique, however serological assessment of IgG and/or IgM against *T. gondii* has been widely used for identifying the infected individual (Villerd *et al.*, 2016; CDC, 2019). To our knowledge there are very few research studies carried out on this important public health issue in Saudi Arabia. This study was therefore planned to fill this research gap and determine the extent of seroprevalence and the associated risk factors in pregnant women, who may act as a potential source of transmission of infection.

MATERIAL & METHODS

Study Design and Population

The current study was conducted in Prince Sattam Bin Abdulaziz University Hospital, AlKharj, Kingdom of Saudi Arabia between November 2017 and November 2018. The study enrolled all pregnant women attending the hospital for ante-natal care irrespective of their trimester of pregnancy. The first trimester is from week 1 through week 12, the second trimester is from week 13 through week 28 and the third trimester is from week 29 through week 40. The study was approved by the ethical committee of Prince Sattam Bin Abdulaziz College of Medicine. The protocol (Protocol no: PSAU/COM/RC/IRB/P/52) and all procedures of the study were conducted in accordance with Good Clinical Practice guidelines and in conformity with the ethical guidelines of the Declaration of Helsinki. All patients submitted a written informed consent before enrollment and before the commencement of any study related procedure.

Significance of risk factors

All patients were explained the risk factors of toxoplasmosis, a disease caused by a protozoan parasite known as *Toxoplasma gondii*. The transmission of this disease is either foodborne, that is, through consumption of raw or undercooked meat contaminated with cysts of parasites (Jones *et al.*, 2006) or contaminated by knives, utensils, cutting boards or other foods that had contact with raw, contaminated meat; or ingesting oocyst through contact with infected cat feces or by touching contaminated soil or consuming food or water contaminated with oocyst (Joyner, 1982); last, but not the least, it could be transmitted from infected mother to fetus through placenta (Alzaheb, 2018).

Data Collection

A consent form was filled by every participant showing willingness to participate in the study. An interviewer administered the structured questionnaire comprising simple, closed ended questions. A template was also used to collect personal details such as age, parity, period of gestation of participants. Such information related to risk exposure such as contact with cats, consumption of undercooked meat, and awareness of the disease was also collected to understand the sample better.

Serological examination for *T. gondii* antibodies

After venipuncture, 5 ml of whole blood was drawn from every participant who had signed the written consent. Sera were kept frozen at -20°C until analyzed. A commercial automated Cobas e411 (Roche Diagnostic GmbH, Mannheim, Germany) *electrochemiluminescence* (ECL) was used to analyze the sera for *T. gondii*. IgG antibodies and *T. gondii* IgM antibodies were tested with a commercially available enzyme immunoassay "*Toxoplasma* IgG" and "*Toxoplasma* IgM" (Roche Diagnostic GmbH, Mannheim, Germany) according to the manufacturer's instructions. Serum levels of either IgG or IgM < 1 IU/mL were considered as negative, $\geq 1-3$ IU/mL were taken as

intermediate and ≥ 3 IU/mL were seen as reactive or positive. Positive and negative controls were already provided by the manufacturer. Hence it was easier to interpret a positive IgG and a negative IgM as a latent infection where as positive IgG and a positive IgM were taken as probability of a recent or acute infection.

Statistical Analysis

The data collection for the enrolled subjects was standardized through the use of similar methodology. Protocol and procedure were used for administering a standard questionnaire. Once data was collected, it was entered into SPSS statistical software, version 24 (IBM, Chicago, Ill, USA) for analysis. The datasets in categorical variables were summarized as proportions and analyzed using the Pearson's Chi-square test to present the difference among various groups. Continuous variables were summarized as mean standard deviation (\pm). Univariate analysis and multivariate logistic regression models were fitted to determine factors associated with infections. These factors were age, residence, occupation, education level, gravidity, eating under-cooked meat and contact with cats and so on. Odd ratios (OR) and their confidence interval [95% CI] were also noted. Factors with the p-value of less than 0.05 on multivariate logistic regression analysis were also considered having a statistically significant association with *T. gondii* infection.

RESULTS

A total of three hundred and six (306) pregnant women attending university hospital aged between 16 and 45 years old (mean age = 29.9 ± 5.78 years) were enrolled in the study and followed up. The demographic characteristics are exhibited in Table 1. Most of the women were from urban residence 259/306 (84.6%), housewives 234/306 (76.5%), multigravida 157/306 (51.3%) and in the third trimester 138/306 (45.1%). The majority of them 149/306 (48.7%) had primary

education up to grade 6, with 125/306 (40.8%) attaining a higher level of education (Table 1). Of the 306 pregnant women, 99 (32.4%) were positive for anti-*T. gondii*-specific IgG antibodies, indicating past infection and only 3 (1.0%) of them were IgM antibody positive (Table 2).

Table 1. Socio-demographic and obstetric characteristics of pregnant women attending an ante-natal clinic at a teaching hospital in Al Kharj, Saudi Arabia (n=306)

| Characteristics | n (%) |
|-----------------------------------|------------|
| Age in years | |
| Mean (SD) | 29.9 (7.2) |
| Residence | |
| Urban | 259 (84.6) |
| Rural | 47 (15.4) |
| Education | |
| Illiterate | 32 (10.5) |
| Primary education (up to Grade 6) | 149 (48.5) |
| More than 10 years of education | 125 (40.5) |
| Occupation | |
| Housewife | 234 (76.5) |
| Others (working women etc.) | 72 (23.5) |
| Obstetric features | |
| Gravidity | |
| Primi-gravida | 102 (33.3) |
| Multi-gravida | 157 (51.3) |
| Grand-multi | 47 (15.4) |
| Trimester | |
| First trimester | 46 (15.0) |
| Second trimester | 122 (39.9) |
| Third trimester | 138 (45.1) |

Table 2. Knowledge, practices and sero-prevalence of toxoplasmosis among pregnant women attending an ante-natal clinic at a teaching hospital in Al Kharj, Saudi Arabia (n=306)

| Findings | n (%) |
|----------------------------------|------------|
| Knowledge about toxoplasmosis | 16 (5.2) |
| Consumption of under-cooked meat | 42 (13.7) |
| Contact with cats | 118 (38.6) |
| Positive IgG | 99 (32.4) |
| Positive IgM | 3 (1.0) |
| Latent infection; (+IgG, -IgM) | 99 (32.4) |

The *T. gondii*-specific IgG antibodies in latent infection were higher in pregnant women who used undercooked meat (13.7%) [OR=1.90[0.98-3.68] p=0.05]. (Tables 3 & 4). The *T. gondii*-specific IgG antibodies were higher in 2nd and 3rd trimester 0.262[.101-.675] p=0.006, .025[0.96-0.634] p=0.004 respectively (Table 4). A total of 118/306 (38.6%) of the women had contact with cats, of whom 47.4% (56/118) tested positive for *T. gondii*-specific antibodies. Thus, the main risk factors for *T. gondii* found in this study were that pregnant women had contacts with cats and consumed undercooked meat. No significant correlation was found between seropositivity and the participants' level of residence, education, occupation, gravidity and knowledge of toxoplasma.

DISCUSSION

Globally there is a wide variation in the prevalence of *toxoplasmosis* among pregnant women (Jula, *et al.*, 2018). In the present study, an attempt was made to determine some of the risk factors and prevalence of *Toxoplasma infection* in pregnant women attending an antenatal clinic at a teaching hospital in Al-Kharj, a rapidly developing city of Saudi Arabia.

The study found evidence of seropositivity for IgG in 99/306 (32.34%) cases with an interestingly increased predilection

for younger age i.e. 41.67% of seropositive females were younger than 26 years of age while the mean age of women participated in this study and attended the antenatal clinic was 29.9 (age range 16-45) years. This finding is consistent with the findings of (Sroka *et al.*, 2010), who discovered an increased prevalence of *toxoplasmosis* in younger age group as compared to older pregnant women. This study however has a few limitations as it primarily focused on lower socioeconomic status significantly associated with such infections. Hence, it contended that the likelihood of infection increased during early adulthood and childhood specifically in a resource-poor population. These findings also explain a high seroprevalence (> 90%) even in very young age groups (Sroka *et al.*, 2010).

Studies in seroprevalence vary on the basis of sampling and inclusion criteria, such as age groups, gender, socioeconomic status, cultural and religious differences and geographical locations. For instance, Sroka *et al.* (2010) study is mainly accountable for higher IgG seroprevalence in younger patients. Sroka *et al.* (2010) found a higher percentage of IgG positive female in all age groups unlike the results of the current study. Contextually speaking and as admitted by authors themselves, Sroka *et al.* (2010) study was biased and limited to a restricted sample as they confined the low prevalence of IgM in their study to lower socioeconomic groups and patients of very young age groups,

Table 3. Association of latent toxoplasmosis infection among pregnant women attending an ante-natal clinic at a teaching hospital in Al Kharj, Saudi Arabia; with their knowledge and practices regarding toxoplasmosis (n=306)

| | Presence of latent toxoplasmosis | Absence of latent toxoplasmosis | P value* |
|---------------------------------|----------------------------------|---------------------------------|----------|
| Knowledge of toxoplasmosis | | | |
| Yes | 6 (6.3) | 10 (4.7) | 0.569 |
| No | 89 (93.7) | 201 (95.3) | |
| Consumption of undercooked meat | | | |
| Yes | 17 (17.5) | 25 (12.0) | 0.226 |
| No | 80 (82.5) | 184 (88.0) | |
| Contact with cats | | | |
| Yes | 58 (61) | 60 (28.4) | 0.000 |
| No | 37 (39) | 151 (71.6) | |

Table 4. Association of risk factors and Seroprevalence of *T. gondii* infection among pregnant women attending an ante-natal clinic at a teaching hospital in Al Kharj, Saudi Arabia (n=306)

| Demographic Characteristics | Ig G (306) | | | | Total | | Univariate OR [95%CI] | p-Value |
|---|---------------|----|----------------|----|-------|------|-----------------------|---------|
| | Positive (99) | | Negative (207) | | No | % | | |
| | No | % | No | % | | | | |
| Age (Years) | | | | | | | | |
| 16-25 | 35 | 35 | 49 | 24 | 84 | 27.5 | 1 | |
| 26-35 | 44 | 44 | 106 | 51 | 150 | 49.0 | 1.77 [0.971-3.21] | <0.05 |
| 36-45 | 20 | 20 | 52 | 25 | 72 | 23.5 | 1.9 [0.923-3.93] | 0.08 |
| Residence | | | | | | | | |
| Urban | 79 | 80 | 180 | 87 | 259 | 84.6 | 1.70 [0.856-3.40] | 0.12 |
| Rural | 20 | 20 | 27 | 13 | 47 | 15.4 | | |
| Education | | | | | | | | |
| Illiterate <10 (less than 10 th grade) | 14 | 14 | 18 | 9 | 32 | 10.5 | 1 | |
| >10 (more than 10 th grade) | 43 | 43 | 106 | 51 | 149 | 48.7 | 1.80 [0.792-4.12] | 0.15 |
| 10 th grade) | 42 | 42 | 83 | 40 | 125 | 40.8 | 1.60 [0.66-3.71] | 0.26 |
| Occupation | | | | | | | | |
| House Wife | 77 | 78 | 157 | 76 | 234 | 76.5 | 1.11 [0.63-1.97] | 0.7 |
| Others (working women, etc.) | 22 | 22 | 50 | 24 | 72 | 23.5 | | |
| Gravidity | | | | | | | | |
| Primary | 38 | 38 | 64 | 31 | 102 | 33.3 | 1 | |
| Multi* | 50 | 51 | 107 | 52 | 157 | 51.3 | 1.22 [0.709-2.12] | 0.46 |
| Grand Multi** | 11 | 11 | 36 | 17 | 47 | 15.4 | 1.87 [0.835-4.21] | 0.12 |
| Trimester | | | | | | | | |
| 1st Trimester (0-12 week) | 6 | 6 | 40 | 19 | 46 | 15.0 | 1 | |
| 2nd Trimester (week 13-27) | 44 | 44 | 78 | 38 | 122 | 39.9 | 0.262 [0.101-0.675] | <0.006 |
| 3rd Trimester (week 28-40) | 49 | 49 | 89 | 43 | 138 | 45.1 | 0.025 [0.96-0.634] | <0.004 |
| Under cooked meat | | | | | | | | |
| Yes | 19 | 19 | 23 | 11 | 42 | 13.7 | 1.90 [0.98-3.68] | <0.05 |
| No | 80 | 81 | 184 | 89 | 264 | 86.3 | | |
| Contact with cat | | | | | | | | |
| Yes | 56 | 57 | 62 | 30 | 118 | 38.6 | 0.32 [0.197-0.545] | <0.0001 |
| No | 43 | 43 | 145 | 70 | 188 | 61.4 | | |
| Knowledge of toxoplasma | | | | | | | | |
| Yes | 8 | 8 | 8 | 4 | 16 | 5.2 | 2.18 [0.79-6.00] | 0.12 |
| No | 91 | 92 | 199 | 96 | 290 | 94.8 | | |

* Multi- refers to a woman pregnant now and has been pregnant one or more times previously.
Grand Multi-refers to a woman who has 6 or more previous pregnancies.

children and teenagers. They have pointed out a high seroprevalence of infection with *T. gondii* in children (40%) and teenagers (60%) in Fortaleza and other parts of Brazil. Our findings are consistent with the findings of other authors from the same region (Al-Harhi, Janjoom, & Ghazi, 2006; Almushait, 2014; Aqeely, El-Gayar & Perveen, 2014; Mohammad, Bahatiq & Degnah, 2016).

IgG positivity is also evident in urban dwellers (30.50%) which is somewhat lower as compared to rural women (42.55%) with a p-value of 0.12, which is statistically not significant. This statistically insignificant difference between the seroprevalence of IgG in urban and rural women may be attributable to improper management, lack of education and awareness, and poor hygiene. These factors are not only specific for toxoplasmosis, but also applicable in other zoonotic diseases in pregnant women specifically (Khan *et al.*, 2019). However, this reflected an increased prevalence of *Toxoplasmosis* in rural dwellers, a well-known fact described by others researchers as well (Sroka *et al.*, 2010).

In our study we could not find statistically significant difference regarding level of education, awareness about *Toxoplasmosis*, occupation and status of gravidity of pregnant women and IgG positivity for *T. gondii*. Similar results are also evident in Julia *et al.* in their study from Africa (Julia *et al.*, 2010). The IgG positivity varied in each trimester. For instance, it was found that the highest percentage of seropositivity for IgG against *T. gondii* was in the third trimester of gestation. Univariate analysis showed a statistically significant p-value of less than 0.0006 and 0.0004 for second and third trimester respectively. This proves that the prevalence of seropositivity is directly related to gestational age, a finding confirmed by several other authors. An interesting fact to note is that at this stage the rate of transmission of disease may be very high, however severity of clinical manifestations is minimal (Sroka *et al.*, 2010).

Consumption of undercooked meat was found to be statistically significant in this study. Out of 306 patients who participated in this study, 42 patients confessed to have

used undercooked meat. Of these 19 patients showed positive IgG against *T. gondii* in their sera and 23 were negative. Univariate analysis showed value of 1.90[0.98-3.68] with p-value of <0.05 which is significant. Similar observation was evident in a study in Brazil (Jones *et al.*, 2006).

Pet keeping is a good hobby but contact with cats has been documented as a strong risk factor for *T. gondii* transmission. In our study, 99 patients were positive for IgG against *T. gondii*, of these 56 patients had the history of contact with cats while 43 had no such history. Univariate analysis shows the value of 0.32[0.197-0.545] with p-value of <0.0001 which is highly significant. A univariate analysis was also done by Aloise *et al.* regarding pet keeping and seroprevalence of toxoplasma infection. They showed p-value of 0.05 and 0.01 for having less than three and more than three cats respectively (Aloise, 2017). This finding is consistent with our findings as well.

CONCLUSION

The Seroprevalence of *T. gondii* antibodies observed in the current study was relatively high among pregnant women in Alkharj, Saudi Arabia. Factors like age, consumption of undercooked meat, contacts with cat were identified as independent risk factors for *T. gondii* infection. The results highlighted the need to raise awareness about *toxoplasmosis*, specifically with regard to the way infections occur so that women can take steps to protect themselves and avoid contracting this parasitic infection. Further research on a large scale is needed to support future public health strategies.

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CONFLICTS OF INTEREST

The authors also declare no conflicts of interest.

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