

Original Research Article

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Seroprevalence and Associated Risk Factors of Toxoplasmosis among Females of Qassim University, Qassim, KSA

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ABSTRACT

Toxoplasmosis has worldwide distribution; however, its prevalence varies within countries and communities in the same region. No studies were conducted on the seroprevalence of *Toxoplasma gondii* infection in Qassim region in Saudi Arabia. Our study aimed to determine the seroprevalence of toxoplasmosis among females in Qassim University, Al-melida campus, Qassim and identify the risk factors associated with Toxoplasmosis. A cross-sectional study, recruiting a random sample of 304 healthy females at Al-melida campus, Qassim was conducted on September 2018. A questionnaire was used to collect sociodemographic data of the participants and establish the risk factors leading to *Toxoplasma* infection. Blood samples were collected from the participants to detect *T. gondii* antibodies namely immunoglobulin G (IgG) using the enzyme-linked immunosorbent assay technique. A descriptive analysis of data was done to correlate between seroprevalence and risk factors. Out of 304 females 247 (81.2%) were negative to *T. gondii* IgG, while 57 (18.8%) were positive. Analysis of risk factors showed statistically insignificant association with *Toxoplasma* seroprevalence. The seroprevalence of *T. gondii* among females in our study is 18.8%. No association between risk factors and seropositivity. For future studies, research on a large sample size is required for more accurate seroprevalence.

Keywords

Seroprevalence,
Toxoplasma gondii,
Risk Factors,
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Introduction

T. gondii is a zoonotic sporozoan parasite, infecting warm blooded animals including humans, the definitive host is the cat (Dubey,

2010). Infection may be congenital or acquired, which can result from the consumption or handling of undercooked or raw meat containing tissue cysts, drinking unpasteurized milk or untreated water, lack of

hand hygiene after direct contact with garden soil (Murray *et al.*, 2013) and blood transfusion. Alternatively, it can result from direct contact with cats or from the ingestion of food contaminated by oocysts excreted in the feces of infected cats (Dubey, 2010; Murray *et al.*, 2013). People at high risk of getting *T. gondii* infection are those who have pets especially cats, and those who live in rural areas.

Prior studies investigated high-risk groups such as pregnant women. Primary infection during pregnancy can cause severe damage to fetus and newborns including stillbirth, abortion and blindness. Immunocompetent individuals infected with *T. gondii* are generally asymptomatic, but in individuals with compromised immune systems such as patients with AIDS, chemotherapy or organ transplantation severe infections may lead to death (Torgerson *et al.*, 2013).

Toxoplasmosis diagnosis may be achieved by serologic tests, polymerase chain reaction and histological examination of infected tissues or by isolation of the organism. Serological diagnosis of *T. gondii* infection relies on testing serum for *T. gondii* IgG and IgM antibodies because Toxoplasmosis manifests no clinical signs in 80% of cases in immunocompetent patients (Florence Robert-Gagneux *et al.*, 2012; Fuccillo, 1987). Initial evaluation in the immunocompetent patients involves screening for IgG antibodies that indicates previous infection and IgM antibodies for the recent one, which is best measured by enzyme-linked immunosorbent assay (ELISA) method (Montoya and Liesenfeld, 2004). Polymerase chain reaction (PCR) has been developed to detect *Toxoplasma* DNA in clinical samples (Bastien, 2002).

It has been reported that approximately one third of the world's population is infected

with *T. gondii* (Torgerson *et al.*, 2013). However, this high prevalence varies from country to country (from 0-80%), even across different communities within the same area. In a Pakistani study of pregnant women seroprevalence of *T. gondii* was found to be 19.4% (Nazir *et al.*, 2017). In Egypt during 2013, 2014 a case control study showed that the seroprevalence of toxoplasmosis in high-risk pregnancy group was 50.8% and the low risk group was 5% (Kamal *et al.*, 2015), while in Lebanon, a retrospective cohort study showed a much higher prevalence of 82.6% (Nahouli *et al.*, 2017). Studies in Saudi Arabia revealed a prevalence of 28.5% in Eastern region (Elsafi *et al.*, 2015), 35% in Makkah (Ghazi *et al.*, 2002), 38.8% in Southwestern region (Almushait *et al.*, 2014) and 38% in Riyadh (Almogren *et al.*, 2011). The most recent study was done in Tabuk University in 2017 which showed that 9.4% were positive for *T. gondii* infection (Alzaheb and Al-Amer, 2017).

Despite the published results showing high prevalence of *Toxoplasma* infections in regions of the kingdom of Saudi Arabia that are socioeconomically similar to Qassim region, no studies have been done in Qassim region. Therefore, there is great demand to determine the seroprevalence of toxoplasmosis among females in Qassim region.

Materials and Methods

A cross-sectional study was carried out in Melida campus at the University of Qassim, Saudi Arabia in 2018-2019. The study population involved apparently healthy female students and employees in the campus.

A random sample has been selected based on standard calculations to represent the population of Melida campus at the University of Qassim, Saudi Arabia. The

representative sample of the population was calculated as shown below:

$$N=Z^2pq/d^2$$

Total population of Al-melida campus is 3339.

Survey questionnaire

Data had been collected directly from the participants by filling a validated questionnaire (Alzaheb and Al-Amer, 2017) which includes questions about sociodemographic data that involves age, marital status, monthly income and residence. We have also collected information to assess the relation between risk factors and *Toxoplasma* infection rate such as eating uncooked meat or contaminated fruit and vegetables, drinking unpasteurized milk or water contaminated with cat feces and exposure to garden soil without proper hygiene. A minor modification has been made on the questionnaire [a question about Grade point average (GPA) was deleted].

Blood sample collection and analysis

Venous blood samples were collected from the participants by well-trained nurses, after they have signed a consent form. Blood samples were immediately transferred to the college research laboratory. At the laboratory sera were separated from blood samples and kept at -20°C till further analysis performed. Sera were tested by an indirect ELISA for detection of *T. gondii* antibodies using SERION ELISA classic *T. Gondii* IgG kit (Institut Viriin/ Serion GmbH; Wurzburg).

The test strips of the SERION ELISA classic microtiter plate are coated with specific antigens of the *T. gondii*. Duplicate of standard sera were included in each run and their optic densities are measured and used in calculating the samples optical densities. SERION ELISA classic *T. gondii* IgG was

shown to have a sensitivity of 98.2% and specificity of 99.4% by the manufacturing company Elisa-antibody.com (2018). For the statistical analysis, the IBM. SPSS version 21.0 was used. Descriptive statistics have been employed to summarize the data. Chi-Square test has been used to determine if there is a significant relationship between two nominal (categorical) variables. Al-Qassim regional review board approval has been obtained for this study before carrying on with it. Participants have signed an informed consent that explained the purpose of the study and the rights of the participant for confidentiality and to withdraw at any time without any obligation towards the study team. Participant's anonymity has been assured by assigning each participant with a code number for the purpose of analysis only.

Results and Discussion

A total of 304 female participants attending or working at university of Qassim aged between 17 and 53-year-old (mean age = 21.95 years, SD: 5.025) were enrolled in this study. Most of participants reside in Buraidah (Figure 1) and their parents attained different levels of education as shown in figure 2. More than 75% of the participants have household income of 10000 SAR or more as detailed in Figure 3.

Out of 304 females tested, 245 (81.2%) were negative to anti *T. gondii* specific IgG antibody, (Negative results with SERION ELISA classic *Toxoplasma* IgG have a concentration of less than 10 IU/μl). While 57 (18.8%) were found to have anti *T. gondii* specific IgG antibody which indicates infection (figure 4). Concentrations of *T. gondii* IgG for the positive samples were calculated from the standard curve and ranged from 10-490 IU/μl (table 1). As illustrated in table 2 there is no clear association between studied risk factors and seropositivity (P values > 0.05).

Table.1 Optical densities (ODs) and concentrations of of *T. gondii* IgG of Positive samples

Sample #	OD	Concentration IU/ μl
2	0.20	12.5
3	0.19	12
5	0.40	32
7	0.38	27
9	0.21	12.5
10	0.20	11
14	0.20	12.5
18	0.21	12.5
22	0.26	20
23	1.27	452
25	1.31	453
26	0.27	20
29	1.04	150
30	0.22	14
42	0.18	10
43	0.21	14
51	0.19	11
52	0.18	10
53	0.24	15
55	0.26	20
58	0.32	25
59	0.19	11
60	0.25	19
64	0.25	19
65	0.99	148
74	0.36	28
77	0.98	148
90	0.22	14
98	0.24	19
99	0.47	36
100	0.43	34
101	0.17	10
107	1.32	480
113	1.00	150
135	0.97	147
136	0.83	96

142	0.22	14
145	0.26	20
155	0.61	55
170	1.04	150
174	0.18	13
184	0.68	65
204	0.21	14
207	0.19	14
216	1.18	280
222	0.89	110
225	0.99	148
227	0.20	14
249	0.17	13
250	0.18	13
253	0.96	147
255	0.30	24
261	0.17	10
263	0.27	20
268	0.99	148
277	1.38	490

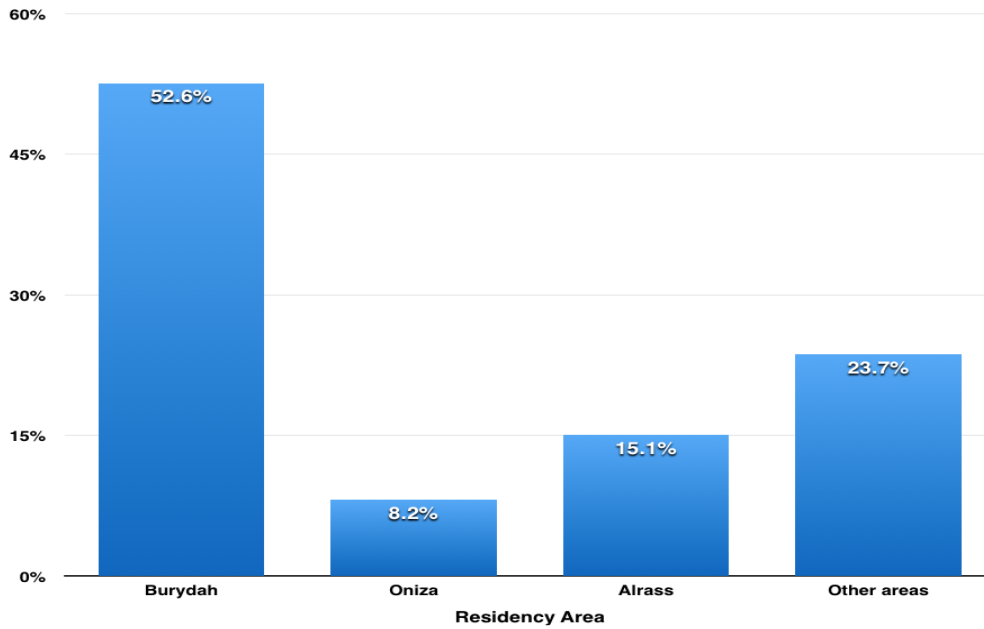


Figure .1 Most of participants reside in cities

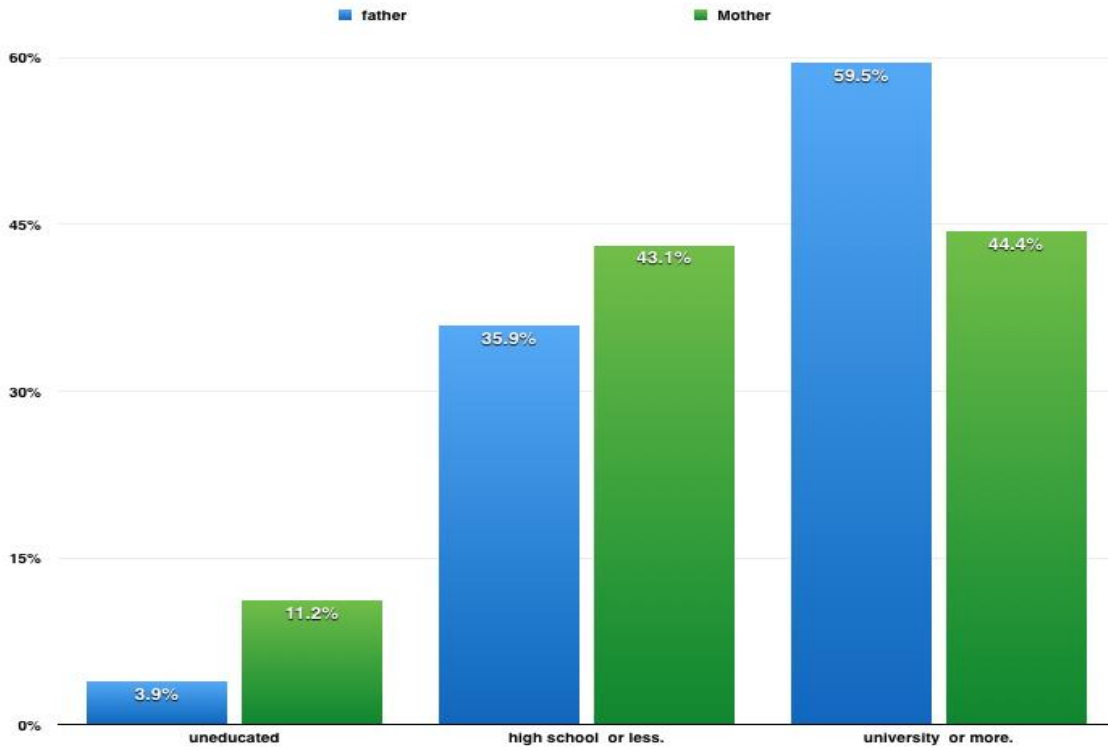


Figure.2 Participants parent's Education

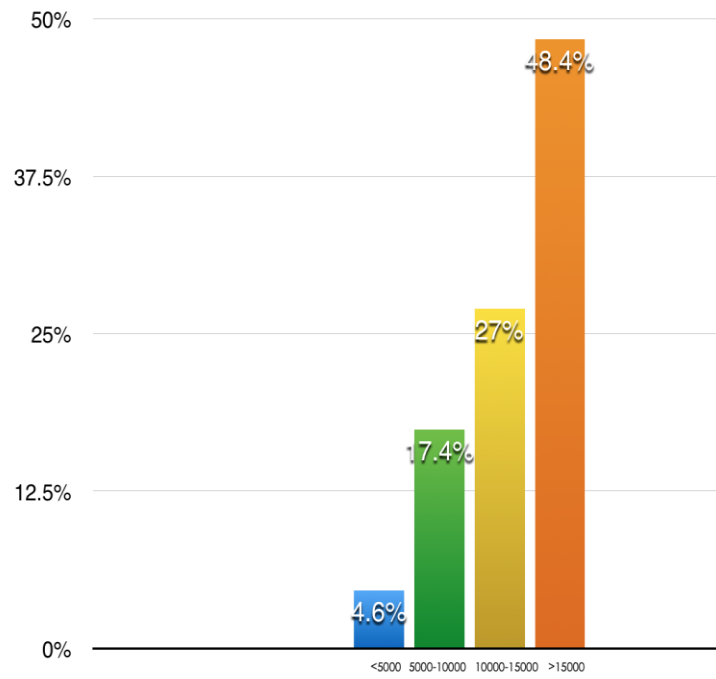


Figure.3 Monthly Income of participants

Table.2 Analysis of the risk factors associated with seroprevalence of toxoplasmosis

IgG	Positive	Negative	P value
Residence in rural areas	20	52	0.131
Monthly income less than 10.000 SAR	12	55	0.720
Ownership of a cat	12	56	0.791
Close contact with cats	14	62	0.956
Consumption of raw or under cooked meat	5	27	0.632
Consumption of unwashed fruit & vegetables	3	21	0.410
Consumption of unpasteurized milk	4	18	0.882
Do not wash after contact with raw meat	4	14	0.673
Contact with garden soil	23	92	0.663
Drinking untreated water	3	25	0.253

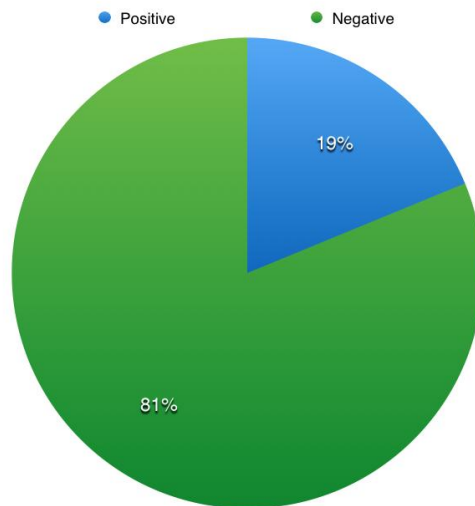


Figure.4 Seroprevalence of *T. gondii* in females

This is the first report of seroprevalence of toxoplasmosis in Qassim Region, Saudi Arabia. The aim was to determine the seropositivity of *T. gondii* infection and to assess the associated risk factors among females in Almalida campus.

The seroprevalence of *T. gondii* infection

among the participants in our study is 18.8%, which is less than toxoplasmosis rate of infection in most regions in Saudi Arabia, where it was found to be 35% in Makkah (Ghazi *et al.*, 2002), 38.8% in Southwestern region (Almushait *et al.*, 2014) and 38% in Riyadh (Almogren *et al.*, 2011).

However, the prevalence in the current study is higher than that reported from Tabuk which was only 9.4% (Alzaheb and Al-Amer, 2017).

Generally, studies conducted in review of *Toxoplasma* infection rate in Saudi Arabia revealed a mean prevalence of around 30%. The difference between this study and prior studies could be due to regional variation such as the climate since dry climate has less infection rates (Dubey, 2010). The difference may be attributed to the fact that our sample does not represent all categories of the region's population as the majority of participants were students. In addition, some risk factors such as eating in restaurants was not included in the questionnaire.

Although some risk factors were insignificant in this study, it has been reported that they were significant in other studies, such as living in rural areas and contact with garden soil. A study in Egypt showed increased risk of infection in rural residents, low socioeconomic status, and consumption of undercooked meat (P-value < 0.05) (Kamal *et al.*, 2015).

The lack of association with cat contact may be because a small number of our participants are in close contact with cats and those who owned cats are well educated about cats' health and risks of contracting infection from cats.

Unexpectedly, in our study there was no significant difference in the seroprevalence of *T. gondii* between participants with high household income and those with low income this may be because of high awareness of hygienic practices among the participating group.

Unpasteurized milk also plays a role in transmission of *Toxoplasma*; it was not significant in this study, as most of the study

population consume pasteurized milk purchased from the grocery stores.

One of the possible routes for transmission of *T. gondii* is handling or consumption of raw meat, some people may get infected through an open wound with the tissue cyst during frequent handling of raw meat of an infected animal especially if they do not wear gloves. Also, ingestion of tissue cyst is possible either from improper washing of the hands or from using contaminated cooking utensils. There is a variation in the significance of this factor among many studies, some suggest a strong relation between the consumption of undercooked meat and *T. gondii* infection such as studies in Brazil, while others found it insignificant in China (P-value = 0.496) and Dhahran (P-value = 0.149) (Elsafi *et al.*, (2015);(Fernandes *et al.*, (2009); Yang *et al.*, (2017)).

Other risk factors such as consumption of unwashed fruit and vegetables and not washing hands after contact with raw meat were insignificant. These factors may have a limited role to play in the region because of the religious and cultural influences that shape dietary habits. However, being insignificant in the current study does not exclude the probability of these factors to affect toxoplasmosis transmission.

In this study we faced some limitations that could be avoided in the future. First, the sample size was calculated as 322 based on the prevalence from previous studies which was around 30%. However, due to time limitation we have included 304, mainly students. Second, ELISA test has been done for anti-*T. gondii* specific IgG antibody that detects infection either previous or current hence further testing such as IgM or polymerase chain reaction will be useful in those who are at risk for complications. Third, the risk factors were evaluated using the study

participants' questionnaire responses, which may have been affected by recall bias to some degree. Finally, this study did not assess all possible risk factors associated with *T. gondii* such as eating in restaurants.

In conclusion the prevalence of *Toxoplasma* infection detected in our study was relatively low compared to most parts of Saudi Arabia and higher than Tabouk. However, the prevalence is significant and further study including pregnant women is necessary considering the risk to the unborn fetuses. Moreover, the results draw attention to the need for raising awareness of toxoplasmosis, specifically with regard to the way infections occur so that women can take steps to protect themselves.

For future studies, research on a large sample size is required for more accurate seroprevalence.

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