Epidemiol. Infect. (2015), **143**, 1898–1903. © Cambridge University Press 2014
doi:10.1017/S0950268814003604

Seroepidemiology and risk factors of *Toxoplasma gondii* infection in undergraduate university female students in Jordan

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Received 18 September 2014; Final revision 25 November 2014; Accepted 30 November 2014; first published online 29 December 2014

SUMMARY

This study estimated the seroprevalence and risk factors for acquiring *Toxoplasma gondii* infection by undergraduate female university students in Jordan. A cross-sectional study from September 2013 to July 2014 analysed 202 blood samples for IgG and IgM antibodies by enzyme-linked immunosorbent assay and a semi-constructed questionnaire was completed by participants to gather information about *Toxoplasma* infection risk factors. *T. gondii* IgG antibodies were detected in 66·5% of the females. Only one sample was positive for both IgG and IgM. Using χ^2 test, six factors showed significant association with *T. gondii* infection ($P \leqslant 0{\cdot}01$). The multivariate logistic regression model showed that female students living in houses, wet areas, with income >US$750/month and using spring (untreated) water were 47·42, 10·20, 5·00, 3·25 more times at risk to be seropositive for *T. gondii*, respectively, compared to female students living in apartments, dry areas, with income ≤US$750/month and using treated water, respectively. This study concluded that *T. gondii* infection in female university students in Jordan is high and most women become infected before marriage; however, congenital toxoplasmosis is still likely to occur in Jordan. Thus, dissemination of protective measures and knowledge by healthcare professionals is essential especially for pregnant women.

Key words: ELISA, females, Jordan, risk factors, seroprevalence, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasmosis is a worldwide zoonotic disease caused by the protozoan parasite *Toxoplasma gondii* [1, 2]. Felids are the definitive hosts, which shed non-infectious oocysts in their faeces [3]. The oocysts become infectious in the environment and remain infective for lengthy periods in water or soil [3, 4]. Many warm-blooded animals and humans serve as intermediate hosts [3, 4].

The prevalence and level of exposure to *T. gondii* in a population is influenced by many factors such as climate, culture, eating habits and animal fauna. *T. gondii* infection in pregnant women may lead to abortion, neonatal death or different congenital defects, such as hydrocephalus, central nervous system abnormalities, chorioretinitis, and reduced intelligence or in some instances to schizophrenia [2, 5–7]. Therefore, serological screening for *T. gondii* antibodies might be conducted in women of childbearing age to identify women at risk of acquiring infection [8] and as

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strategic approach for preventing congenital infection [8]. However, seroprevalence ranges from 4% to 100% in women of childbearing age in different countries around the world [9]. Meanwhile, there is a dearth of information about this disease in several Middle Eastern and North African (MENA) countries such as Jordan, where toxoplasmosis is a neglected zoonotic disease and the level of infection in women of childbearing age is unknown. Therefore, this study aimed to estimate the seroprevalence and potential risk factors hypothesized to be associated with the occurrence of *T. gondii* infection in undergraduate university female students in Jordan.

MATERIAL AND METHODS

Design

A cross-sectional epidemiological study was conducted to recruit undergraduate university students from several faculties at Jordan University of Science and Technology (JUST). Only non-medical female students were recruited in order to study the prevalence and risk factors associated with *T. gondii* infection in childbearing females in Jordan. The students were from different geographical regions since JUST is a leading university in Jordan that offers various medical and non-medical specializations.

Setting

The study was conducted in JUST campus to determine the seroprevalence of *T. gondii* infection in female university students. A random sample of undergraduate university female students was recruited. Sampling recruitment was conducted through collaboration with the Deanship of Student's Affairs (DSA) of JUST. A list of the non-medical female students (target population) was obtained from the SDA and stratified according to the location of residency (wet and dry). These two strata were entered separately in the SPSS random generator model. A weighted random sample from each stratum was obtained using a simple random sampling method to ensure representativeness of subjects in these regions of Jordan. All eligible female students were invited to participate and approached by the investigators during classes and briefed about the study's aims. Female students, who agreed to participate in the study, were asked to sign a consent form detailing the aims of the study and explaining the students' right to withdraw from the research at any time without penalty. Participants were asked to complete a questionnaire written in Arabic to gather information and practices related to the occurrence of *T. gondii* infection.

Sample size

A total of 202 students (aged 18–23 years) voluntarily completed the questionnaire and provided blood samples at the student's clinic on campus. Sample size was calculated based on a power analysis of 0·8, expected *Toxoplasma* prevalence rate of 50% and level of significance (alpha-error) of 0·05 and a precision of 0·06 around the true population mean [10]. The minimum required sample size needed for this study to examine the seroprevalence of *T. gondii* infection was 196 students; however, 202 students provided blood samples.

Risk factors associated with *T. gondii infection*

A checklist accompanying the self-report semi-structured questionnaire (written in Arabic, available from the corresponding author upon request) was developed to gather information about factors hypothesized to be associated with sociodemographic characteristics of participating undergraduate female students (Table 1). These risk factors were identified based on the published literature [1–4, 6]. A pilot testing of the questionnaire was performed using 20 participating and five non-participating female students and all necessary revisions were made to the final form of the questionnaire. Repeatability of the questionnaire was examined by having some participants complete the questionnaire on two different occasions and their repeatability was estimated using the kappa (κ) statistic. The external validity was tested using records of the DSA.

Blood sample collection and diagnosis of *T. gondii* infection

A single blood sample was withdrawn from each participating student using sterile plain Vacutainer tubes (BD Vacutainer Systems, UK) by a registered nurse. All collected blood samples were transported to the laboratory within 4 h of collection and were centrifuged at 3000 rpm for 10 min to harvest the serum. The sera samples were placed in Eppendorf tubes and stored at −20 °C until analysis.

All samples were serologically screened for *T. gondii* for IgG and IgM antibodies using enzyme-linked immunosorbent assay (ELISA) kits (Novatec,

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Table 1. *Risk factors for seropositivity to* Toxoplasma gondii *in undergraduate university female students at Jordan University of Science and Technology, 2013–2014*

Variable	Seropositive (%)	χ^2 (*P* value)
Cat-ownership		0·204
No	12	
Yes	7	
Housing		**0·001**
Apartment	52	
House	92	
Residency		**0·001**
City	49	
Village	73	
Family income/month (US$)		**0·014**
⩽750	45	
>750	63	
Drinking water		**0·015**
Tap	37	
Untreated spring	49	
Wild traditional herb consumption		**0·016**
No	58	
Yes	75	
Meat cooking consumption		0·081
Rare	86	
Well done	74	
Hand washing after meat handling		0·091
No	70	
Yes	81	
Location (area)		**0·001**
Dry (dry <300 mm rainfall/year)	57	
Wet dry ⩾300 mm rainfall/year	92	

Germany). All runs were conducted according to the manufacturer's instructions. Negative and positive controls were tested in each run. A positive IgG and a negative IgM result for each participant was considered as a latent infection. Detection of both IgG and IgM antibodies in blood serum was interpreted as a recent or acute infection.

Ethical considerations

This research was approved by the Institutional Research Bioethics committee of JUST (IRB no. GM7601). Before completing the questionnaire, all participants were briefed about the study objectives and written informed consent was obtained from all participants. It was emphasized to all participants that providing a blood sample is done voluntarily.

Table 2. *Final multivariate logistic regression model for seropositivity to* Toxoplasma gondii *in undergraduate university female students at Jordan University of Science and Technology, 2013–2014*

Variable	*P* value	OR	95% CI
Housing	0·001	47·42	15·62–143·92
Location (area)	0·001	10·20	4·05–25·69
Family income/month	0·001	5·00	1·99–12·57
Drinking water	0·005	3·25	1·42–7·46

OR, Odds ratio; CI, confidence interval.
Hosmer & Lemeshow test: $\chi^2 = 6{\cdot}97$, D.F. = 7, $P = 0{\cdot}432$.

The names of blood donors were maintained in a confidential log and each blood sample was assigned a number from 1 to 202. Results of the test were sent to each participant confidentially by secure university email.

Data management and statistical analysis

The data were entered into a Microsoft Excel spreadsheet (Microsoft Corp., USA) and analysis was performed using IBM SPSS v. 20·0 software for Windows (IBM SPSS Corp., USA). Associations between seropositivity for *T. gondii* infection (outcome variable) and its potential risk factors were first screened in a univariable analysis using χ^2 test. Potential risk factors with $P \leqslant 0{\cdot}25$ (two-tailed, $\alpha = 0{\cdot}25$) and no collinearity ($r < 0{\cdot}6$) were considered for further analysis (Table 2). Collinearity between potential risk factors was examined pairwise using Spearman's rank correlation test. The overall seroprevalence rate was calculated as the total number of serologically positive samples by the total number of all tested samples.

A multivariable model for seropositivity of *T. gondii* infection was constructed using manual stepwise forward logistic-regression analysis. Risk factors that were not significant in the model were re-entered whenever a new risk factor became significant, or a risk factor was removed. Potential confounders were considered in every model. A risk factor was considered as a confounder if its coefficient was statistically significant at $P < 0{\cdot}10$ (two-tailed) or the point estimates of the coefficients in a model changed >20% with the potential confounder present [11]. Possible interactions between some potential risk factor were evaluated in the final models. The fit of the models was evaluated using the Hosmer & Lemeshow goodness-of-fit test [12].

https://doi.org/10.1017/S0950268814003604 Published online by Cambridge University Press

RESULTS

The point seroprevalences of IgG and IgM antibodies was 66·5% and 0·5%, respectively. χ^2 test results of potential risk factors of *T. gondii* infection seropositivity are shown Table 1. χ^2 test revealed that housing type ($P = 0{\cdot}001$), residency location ($P = 0{\cdot}001$), family income ($P = 0{\cdot}014$), drinking water source ($P = 0{\cdot}005$), consumption of raw wild traditional herbs ($P = 0{\cdot}016$) and study area ($P = 0{\cdot}001$) were all risk factors for acquiring *T. gondii* infection.

The final model of the multivariate logistic regression is shown in Table 2 and included only four independent risk factors that were statistically significant ($P \leqslant 0{\cdot}05$). After adjusting for the other three risk factors in the model, the multivariate logistic regression model showed that the probability of acquiring *T. gondii* by women living in houses was 47·42 times greater ($P = 0{\cdot}001$) compared to women live in apartments; women living in wet areas were 10·20 times more likely to acquire infection than women in dry areas; and women who drank and used spring (untreated) water were 3·25 times more likely to become infected compared to those who did not. Women with >US$750/month family income were also 5·0 times more likely to become infected than women with lower family income (Table 2).

DISCUSSION

In this study, only one female student was seropositive (IgM) for to *T. gondii* infection which suggested a recent infection. The seroprevalence rate of *T. gondii* infection (IgG) in female university students was 66·5%. This is much higher compared to other countries around the world. Seroprevalence in USA, Portugal and Greece in women of childbearing age was 11·0%, 24% and 20%, respectively [13–15]. This low prevalence makes women in these countries at lower risk of developing congenital toxoplasmosis [16]. The seroprevalence for Arabs living in Israel (non-Bedouins) was 72·3% in women of reproductive age, but much lower in Bedouins (25·4%) and Jews (15·1%) [17]. High seroprevalence was reported in other countries such as Brazil (64·9%) [18] and Ethiopia (81·4%) [19]. The overall seroprevalence of *T. gondii* infection in the general population in Iran was 39·3% [20]. The high seroprevalence of *T. gondii* infection in these countries puts women at higher risk of acquiring *T. gondii* infection during pregnancy with a risk of congenital transmission [16]. This necessitates educating women, specifically those most vulnerable, about the risk and prevention of *T. gondii* since education is associated with reduction in toxoplasmosis seroconversion [21].

The importance of the different risk factors for contracting toxoplasmosis differs between countries and a given population in a country due to differences in cultural habits and climatic factors that affect oocyst survival, cat densities and the presence of stray cats that have access to areas where they can contaminate feed and water with oocysts [4, 22, 23]. In the USA, multiple risk factors for toxoplasmosis infection were identified including consumption of raw ground beef, rare lamb meat, locally produced cured, dried, or smoked meat, raw clams, oysters and mussels, working with meat, drinking unpasteurized goat's milk, owning three or more kittens [24] and exposure to cat faeces [25]. In Mexico, consumption of turkey meat was identified as risk factor [26]. In Portugal, women who engage in soil-related activities without wearing gloves, eat unwashed raw vegetables or fruit, and consume smoked or cured (non-cooked) processed pork products were identified at higher risk for contacting toxoplasmosis than women who do not [14]. In Korea, consumption of raw or undercooked meat is the major route of toxoplasmosis infection [27]. Boyer *et al.* [25] reported that indirect or direct exposure to raw or undercooked meat or to cat faeces were risk factors in mothers of infants with congenital toxoplasmosis in the USA.

Specifically in this study, women living in houses were 47·42 times more likely to acquire *T. gondii* infection ($P = 0{\cdot}001$) than women living in apartments as shown by the multivariate analysis. This might be attributed to owning backyard gardens by those women where they grow vegetables and traditional herbs such as dandelion, mint and sage. These vegetables and herbs might have exposure to oocytes from infected stray cats. Similar finding were reported in Turkey [28] and Portugal [14]. Interestingly, students from families with income >US$750/month were at higher risk of being seropositive to *T. gondii.* This might be attributed to fact that people in Jordan with high income are more likely to live in houses with backyards. Specifically, this study revealed that about two thirds of families with high incomes live in houses.

The multivariate analysis showed that women living in wet areas were 10·20 times more likely to acquire infection than women in dry areas. The higher seroprevalence of *T. gondii* infection in women in wet areas compared to dry areas might be attributed to

https://doi.org/10.1017/S0950268814003604 Published online by Cambridge University Press

the longer survival of oocytes in humid areas. This phenomenon was also reported in Israel, Iran, and Niger [17, 29, 30].

Consumption of raw vegetables significantly increased the seroprevalence of *T. gondii*; where infection was greater in individuals who ate raw vegetables than those who did not. In our study, univariante analysis showed that eating wild traditional herbs can be a risk factor for *T. gondii* infection. This might be due to the fact that oocysts from stray cat faeces can contaminate these plants and other vegetable products. Kapperud *et al.* [22], Njunda *et al.* [31] and Liu *et al.* [32] reported that eating raw vegetable is an important risk factor for acquiring *T. gondii* infection in Norway, Cameroon, and China, respectively. Prepacked ready-to-eat fresh vegetables are probably at lower risk, but these products have been shown to be contaminated with pathogenic microorganisms and may pose a theoretical risk of toxoplasmosis [33]. The access of stray cats to vegetable fields, and lack of oocyst eradication measures during vegetable processing might provide the opportunities for contamination and survival of *T. gondii* oocysts.

Univariate and multivariate regression analyses identified drinking or using spring (untreated) water as a risk factor for acquiring *T. gondii* infection, where women who drank and used spring (untreated) water were at 3·25 times higher risk of infection compared to those who did not. In this way, waterborne toxoplasmosis outbreaks can be associated with untreated water, and theoretically consuming seafood from contaminated water may pose a risk [20, 34]. Seroprevalence was significantly higher in females who drank untreated spring water than in those who did not ($P = 0{\cdot}005$), indicating contamination of spring water by oocysts from stray cats faeces, which has been confirmed by other studies [1, 30, 35, 36].

Felids are the only definitive hosts which shed non-infectious *T. gondii* oocysts in the environment, the oocysts then sporulate and become infective for lengthy periods [37]. This study did not find any association between *Toxoplasma* seropositivity and cat-ownership. However, there is a debate in the literature regarding the role of seropositivity and cat-ownership. For example, Jones *et al.* [24] in the USA and Gebremedhin *et al.* [19] in Ethiopia reported strong association of seroprevalence with the presence of domestic cats in the household. One the other hand, Kapperud *et al.* in Norway [22], Sroka *et al.* [36] in Poland, Hofhuis *et al.* in The Netherlands [38] and Cook *et al.* [39] in seven large European cities reported that cat-ownership does not associate with *Toxoplasma* seropositivity.

However, recent epidemiological studies identified several risk factors such as cat-ownership, eating raw or uncooked pork, lamb, mutton, beef, game or mincemeat products, eating raw or unwashed vegetables or fruits, poor hand hygiene, infrequent washing of kitchen knives, cleaning the cat litter box, and contact with soil [37, 39, 40]. Meanwhile, outbreaks of toxoplasmosis have been associated with eating raw or uncooked pork, lamb, mutton or beef products [41, 42] and additionally, with oocyst-contaminated water [43].

In conclusion, a very high seroprevalence of *T. gondii* infection was found in female university students in Jordan. This high prevalence might be attributed to the lack of awareness about the disease. This study reports for the first time from Jordan that there is a significant association between *T. gondii* infection and income, drinking and using spring (untreated) water, housing type and climate. The presence of one sample positive for IgM indicates that most females in Jordan acquire *T. gondii* infection at a young age, but there is still a considerable number of women at risk of developing congenital toxoplasmosis in their infants. Therefore, dissemination of appropriate knowledge by healthcare professionals to prevent infection is essential especially for the most vulnerable, i.e. pregnant women.

ACKNOWLEDGEMENTS

This research was funded by the Deanship of Research (Project no. 197/2013) at Jordan University of Science and Technology.

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