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The Seroprevalence and Risk Factors of Toxoplasmosis Among Female Undergraduate University Students in Saudi Arabia

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ABSTRACT

Objectives: *Toxoplasma gondii* (*T. gondii*) is a serious public health issue, but limited data has been published to date on the seroprevalence of *T. gondii* infection in Saudi Arabia. Therefore, this study aimed to establish the seroprevalence and risk factors of *T. gondii* infection using a sample of females enrolled at a university in Northern Saudi Arabia. ***Methods***: Using a cross-sectional research approach, we recruited a convenience sample of 180 healthy females studying at the University of Tabuk between February and June 2016. We used a questionnaire to gather sociodemographic data on the participants to establish the risk factors leading to exposure to toxoplasma. Blood samples were taken from the participants and analyzed to detect *T. gondii* antibodies (immunoglobulin M and immunoglobulin G) via the enzyme-linked immunosorbent assay technique. Multivariate logistic regression modeling was employed to establish the potential predictor variables for *T. gondii* infection. ***Results:*** Of the 180 participants, 17 (9.4%) were seropositive for *T. gondii* IgG. None tested positive for IgM antibodies exclusively. The sole variable positively associated with seropositive *T. gondii* IgG was the participants' consumption of raw, unwashed fruit or vegetables (odds ratio = 3.36, 95% confidence interval: 1.11–10.22). ***Conclusions:*** Our findings emphasize the need for higher awareness of toxoplasmosis in Saudi Arabia, specifically knowledge of the way toxoplasma infection occurs so that women can more easily avoid it. Large-scale research is therefore required to inform the design of future public health interventions.

Toxoplasmosis is a zoonosis with a cosmopolitan distribution caused by intracellular protozoa *Toxoplasma gondii* (*T. gondii*).[1] It can infect numerous mammals including humans, who are mainly infected by three routes. The first route is food-borne transmission, where a human is infected by eating raw or undercooked meat contaminated with the parasitic cyst.[2–4] The second route is animal-to-human, where a human is infected by ingesting oocysts via contact with infected cat feces, or by touching contaminated soil or consuming food or water contaminated with oocysts.[5] The third route is congenital transmission.[6]

It has previously been estimated that approximately one-third of the world's population suffers from *T. gondii* infection, but despite this high worldwide prevalence, there are large variations in the seroprevalence from country to country (from 0 to 80%), within the same country, and even across different communities.[7,8] Saudi Arabia's seropositivity rate has significant regional variations, ranging between 25.0% and 52.1% in different parts of the country.[9,10] These variations may be due to anthropogenic factors including dietary habits (different meats consumed and their cooking methods, vegetable and hand washing practices); social, economic, cultural patterns, quality of local water, and availability of adequate sanitation.[11] Building an understanding of these potentially influential factors is important, because *T. gondii* has a considerable negative impact on human health, and can cause serious outcomes in immunocompromised patients and pregnant women.[7]

A comprehensive knowledge of the possible sources of this infection in specific communities

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would be valuable in planning efficient public health interventions, including those focusing on safety and handling of food.[12] To the best of our knowledge, there is no documented data on the seroprevalence and risk factors of *T. gondii* infection in Northern Saudi Arabia, and few studies have been performed in other parts of the country. Prior studies have mainly investigated high-risk groups such as pregnant women, but have not gathered the basic data needed to develop a full control strategy aimed at the treatment and prevention of *T. gondii* infection. Therefore, we sought to estimate the seroprevalence of toxoplasmosis and identify its possible risk factors among a sample of female university students in Northern Saudi Arabia. The results of this work can be used to support the development of public health policies against toxoplasma infection.

METHODS

We employed a cross-sectional study design and recruited a sample of 180 apparently healthy female students studying at the University of Tabuk, Saudi Arabia. The optimal sample size was worked out using the following assumptions: π = 0.5 (no previous estimate of prevalence of *T. gondii* in Saudi Arabia's Northern province), desired marginal error = 0.075 and/or (confidence level 95%) = 1.96, thus producing a sample size of 171 participants, which the authors approximated to 180 participants. Convenience sampling was therefore used for data collection at various locations on the university campus, all chosen for their convenience in allowing students from across the university's various colleges to participate in the research. The data collection was performed between February and June 2016. All students received a full orientation on the research (including their right not to take part) and were sent forms to return confirming their informed consent to participate. Ethical approval was obtained from the University of Tabuk's Committee of Research Ethics.

We designed a structured questionnaire to examine the known risk factors affecting toxoplasmosis, by gathering sociodemographic data on the participant's age, marital status, residence, and monthly household income. In addition, behavioral and lifestyle information was collected (i.e., contact with cats, consumption of raw or undercooked meat, consumption of raw unwashed fruit or vegetables, drinking unpasteurized milk and/or untreated water, hand washing after preparing raw meat, and exposure to garden soil).

Each participant was asked to give a sample of approximately 5 ml of venous blood. These samples were transported to the clinical laboratory at the University of Tabuk in cool boxes. Serum was separated from whole blood samples by centrifugation for 10 minutes at 3000 rpm at room temperature. The resulting separated serum was then carefully labeled and stored at -20 °C before being tested for anti-Toxoplasma immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies using standard enzyme-linked immunosorbent assay (ELISA) commercial kits (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Germany) in line with the kit manufacturer's guidance. The cut-off value was given as an index. The result of the test was deemed to be negative if the index value was < 0.77, and positive if it was > 0.97 for IgM. Similarly, in terms of cut-off values for IgG detection, < 0.3 was considered negative, and ≥ 0.6 was positive. The manufacturers offer 94.6% sensitivity, and 94.1% specificity.

The data gathered via the questionnaire and laboratory testing was entered into SPSS Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) for analysis. Descriptive statistics was employed to summarize the data. The level of statistical significance was set at a *p*-value of < 0.050. A univariate logistic regression was then carried out, primarily to identify the significant variables for use in a multivariate logistic regression analysis. The odds ratio (OR) and 95% confidence interval (CI) were employed to establish the strength of any associations.

RESULTS

A total of 180 female students attending university aged between 19 and 25 years old (mean age = 21.6 years) were enrolled in this study. Out of 180 students, 17 (9.4%) had a positive *T. gondii*-specific IgG antibodies which indicate previous infection. All participants were negative for *T. gondii*-specific IgM antibodies [Table 1].

None of the following factors were found to be associated with seropositivity: age (OR = 0.82, 95% CI: 0.30–2.22), marital status (OR =

Table 1: Univariate logistic regression analysis of the variables associated with the seroprevalence of toxoplasmosis among the sample (n = 180).

Variables	Toxoplasmosis seropositivity		Unadjusted		*p*-value
	Yes (n = 17, 9.4%)	No (n = 163, 90.5%)	OR	95% CI	
Age category, years					0.690
19–22	8 (8.6)	85 (91.3)	0.82	0.30–2.22	
> 22–25	9 (10.3)	78 (89.6)	1.00	Ref	
Marital status					0.213
Single	5 (6.3)	74 (93.6)	0.50	0.17–1.49	
Married	12 (11.8)	89 (88.1)	1.00	Ref	
Place of residence					0.099
Rural	9 (14.5)	53 (85.4)	2.34	0.85–6.39	
Urban	8 (6.7)	110 (93.2)	1.00	Ref	
Monthly household income, SR					0.302
< 5 000	5 (16.6)	25 (83.3)	3.40	0.61–18.97	
5 000–15 000	10 (8.7)	104 (91.2)	1.64	0.34–7.83	
> 15 000	2 (5.5)	34 (94.4)	1.00	Ref	
Ownership of cat					0.023
Yes	3 (33.3)	6 (66.6)	5.61	1.26–24.88	
No	14 (8.1)	157 (91.8)	1.00	Ref	
Close contact with cat					0.126
Yes	3 (21.4)	11 (78.5)	2.96	0.74–11.88	
No	14 (8.4)	152 (91.5)	1.00	Ref	
Consumption of raw or undercooked meat					0.106
Yes	6 (16.6)	30 (83.3)	2.42	0.83–7.06	
No	11 (7.6)	133 (92.3)	1.00	Ref	
Consumption of raw, unwashed fruit or vegetables					0.007
Yes	10 (19.2)	42 (80.7)	4.12	1.47–11.5	
No	7 (5.4)	121 (94.5)	1.00	Ref	
Consumption of unpasteurized milk					0.075
Yes	5 (19.2)	21 (80.7)	2.82	0.90–8.81	
No	12 (7.7)	142 (92.2)	1.00	Ref	
Handwashing after contact with raw meat					0.142
Yes	9 (7.2)	115 (92.7)	1.00	Ref	
No	8 (14.2)	48 (85.7)	2.13	0.78–5.85	
Contact with garden soil					0.079
Yes	4 (21.0)	15 (78.9)	3.04	0.88–10.49	
No	13 (8.0)	148 (91.9)	1.00	Ref	
Drinking untreated water					0.173
Yes	4 (17.3)	19 (82.6)	2.33	0.69–7.89	
No	13 (8.2)	144 (91.7)	1.00	Ref	

Data presented as n (%). CI: confidence interval; OR: odds ratio; Ref: reference; SR: Saudi Riyal (≈0.266 $US).

0.50, 95% CI: 0.17–1.49), residence (OR = 2.34, 95% CI: 0.85–6.39), monthly household income (OR = 3.40, 95% CI: 0.61–18.97 and OR = 1.64, 95% CI: 0.34–7.83 for income < 5 000 and 5 000–15 000 Saudi Riyal, respectively), contact with cats (OR = 2.96, 95% CI: 0.74–11.88), the consumption of raw or undercooked meat (OR = 2.42, 95% CI: 0.83–7.06), drinking unpasteurized milk (OR = 2.82, 95% CI: 0.90–8.81), drinking untreated water (OR = 2.33, 95% CI: 0.69–7.89), hand washing after handling raw meat (OR = 2.13, 95% CI: 0.78–5.85), and exposure

Table 2: Multivariate logistic regression analysis of the variables associated with the seroprevalence of toxoplasmosis among female university students (n = 180).

Variables	Adjusted		*p*-value
	OR	95% CI	
Ownership of cat			0.249
Yes	2.57	0.52–12.80	
No	1	Ref	
Consumption of raw, unwashed fruit or vegetables			0.033
Yes	3.36	1.11–10.22	
No	1	Ref	

CI: confidence interval; OR: odds ratio; Ref: reference.

to garden soil (OR = 3.04, 95% CI: 0.88–10.49). However, cat ownership (OR = 5.61, 95% CI: 1.26–24.88) and the consumption of raw, unwashed fruit or vegetables (OR = 4.12, 95% CI: 1.47–11.5) did have significant associations with seropositivity. These two factors were entered into multivariate analysis which indicated that only the consumption of raw, unwashed fruit or vegetables was a potential risk factor in relation to *T. gondii* infection (OR = 3.36, 95% CI: 1.11–10.22) [Table 2].

DISCUSSION

The present research is the first performed in Northern Saudi Arabia aiming to determine rates of *T. gondii* infection and its associated risk factors among young women.

The study's results reveal a seroprevalence of 9.4% of *T. gondii*-specific IgG antibodies among young female university students, which is less than the seroprevalence previously found in other regions of Saudi Arabia. For example, a 28.5% seroprevalence was recently observed in the Eastern province; 35.6% in Makkah; 38.8% in the Southwestern province; and 38.0% in Riyadh.[13–16] The notable difference between the seropositivity rate of this study and those in prior studies in other parts of the country may be due to the different age groups of the women participating in these studies. Our study sample had a younger mean age than the previous studies.

A recent systematic review of studies investigating seroepidemiology and *T. gondii*'s potential risk factors in Arab and African countries found the seroprevalence of *T. gondii* to be significantly associated with a woman's age.[9] For example, Mwambe et al,[17] established that a woman's risk of infection with *T. gondii* goes up by 7% for each year of her age. One possible reason for this finding is the additional years of potential exposure with age.[18]

However, the seroprevalence rate reported here and the rates found by prior research in Saudi Arabia are much lower than those reported in other countries in or near the Middle East, including Yemen (45.4%), Jordan (47.1%), Iran (75.7%), and Ethiopia (85.4%).[19–22] The high seroprevalence variations between Saudi Arabia and the other countries mentioned here could be due to differences in climate, personal hygiene customs, local dietary practices, and the socioeconomic status and literacy of the subjects in each sample.[22–27]

Of the various risk factors analyzed by our research, the consumption of raw, unwashed fruit or vegetables was the only one to be positively associated with toxoplasmosis. This factor was also identified as significant by other researchers.[25,28–33] It has also been suggested in some studies to be the product of a contaminated local environment (i.e., polluted soil and water) containing oocysts.[12,25,34,35] Sporulated oocysts can persist for years in moist soil, and poor sanitation involving the use of polluted water to wash fruit and vegetables sold at the market may lead to the contamination of those foods with oocysts.[25,36] Therefore, it is important that awareness of how toxoplasma infections occur is raised so that women can take steps to avoid contracting this parasitic infection.

Other risk factors (i.e., age, marital status, residence and monthly household income, contact with cats, consumption of raw or undercooked meat, drinking unpasteurized milk and/or untreated water, hand washing after preparing raw meat, and exposure to garden soil) were not found to have a significant association with the seroprevalence of *T. gondii*. In line with this, a recent study in the Saudi Arabia also confirmed the absence of any statistically significant association between the prevalence of toxoplasma infection among pregnant women in Jazan, a city in Saudi Arabia, and most of the risk factors included in the present research.[37]

The present findings do not exclude the possibility that these factors might influence toxoplasmosis transmission; however, they may have a limited role to play in the region because of the dominant

religious and cultural influences that shape dietary habits.[17,38] For example, a risk factor which has been linked with toxoplasma infection in pregnant Ethiopian women is the consumption of raw or undercooked meat, because Ethiopia has a long and still popular tradition of dining on raw meat, known locally as 'Kurt' in the Amharic language.[27]

The results of the present study should be interpreted cautiously due to some limitations. Firstly, the sample size was relatively small, and different results may have been obtained from a larger sample of participants. Secondly, the risk factors were assessed using the study participants' questionnaire responses, which may have been affected by recall bias to some degree. Thirdly, because this research was institution-based, it may not be regarded as fully representative of the wider population. Fourthly, because *T. gondii* IgG can be a lifelong condition, a person's changing behavior and dietary habits over time may mean that a cross-sectional study such as this one may not capture the behavior and diet when the infection was contracted. Fifthly, the choice of a cross-sectional study means that causal inferences cannot be drawn from the associations identified between the influencing factors and toxoplasma. Finally, this study did not examine all possible risk factors associated with *T. gondii* such as eating outside in restaurants.

CONCLUSION

The seroprevalence of *T. gondii* antibodies observed in our study was relatively low among the sample of female university students in Northern Saudi Arabia. The consumption of raw, unwashed fruit or vegetables was identified as an independent risk factor for *T. gondii* infection. The results highlight the need to raise awareness of toxoplasmosis, specifically with regard to the way infections occur so that women can take steps to protect themselves and avoid contracting this parasitic infection. Research on a large scale is needed to support future public health strategies.

Disclosure
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