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ORIGINAL ARTICLE

SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH *Toxoplasma gondii* INFECTION AMONG RURAL COMMUNITIES IN NORTHERN IRAN

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SUMMARY

Toxoplasmosis is the fourth most common cause of hospitalization and the second cause of death due to food-borne infections. We conducted a cross-sectional study to determine the prevalence, disease awareness and risk factors associated with toxoplasmosis among rural communities in Northern Iran. Data were obtained from serological testing and from participant's questionnaires and were analyzed using a logistic regression. Of the 630 participants, 465 (73.8%), and 12 (1.9%) had IgG and both IgG and IgM anti-*Toxoplasma gondii* antibodies, respectively. In the logistic regression analysis, *T. gondii* seropositivity was associated with the following factors: age, occupation, consumption of undercooked meat, and of unwashed raw vegetables or fruits ($p < 0.001$). Our study showed a high prevalence of *T. gondii* infection in the general population of Northern Iran. A health program is needed to increase the public awareness of toxoplasmosis, and its associated risk factors.

KEYWORDS: Toxoplasmosis; Seroprevalence; Cross-sectional study; Rural communities; Northern Iran.

INTRODUCTION

Toxoplasma gondii infection causes a zoonotic disease known as toxoplasmosis with a worldwide distribution. *T. gondii* infects approximately 30 to 50% of the human population in both developed and developing countries[1]. Toxoplasmosis is the fourth most common cause of hospitalization and the second cause of death due to food-borne infections in the United States[2]. The main risk factors for *T. gondii* infection among humans are consumption of raw or undercooked meat containing *T. gondii* tissue cysts, intake of sporulated oocysts from soil, water, unwashed vegetables or contaminated hands, and vertical transmission of infection through the placenta[3].

Although primary acquired infection is mild and frequently self-limited in adults with normal immune function, exposure to *T. gondii* during pregnancy can lead to ocular and neurological impairment such as mental retardation, blindness, epilepsy, seizures, microcephaly and hydrocephaly[3,4]. Toxoplasmosis is responsible for encephalitis, brain abscesses and death when it is reactivated in immunocompromised patients[3,5,6]. Some recent studies have reported that latent toxoplasmosis could be associated with neuropsychiatric disorders such as schizophrenia, Parkinson disease, suicide, bipolar disorder, obsessive compulsive disorder and anxiety[7,8]. Moreover, some studies reported that latent *T. gondii* infection is related to male and female infertility[9,10]. Therefore, antibody screening in different groups of patients to identify those who are at risk of acquiring a primary *Toxoplasma* infection leading to an increment of epidemiological data on toxoplasmosis in the general population can be helpful to establish control measures and prevention of toxoplasmosis complications[3,11].

A number of seroprevalence studies on toxoplasmosis were carried out in Iran, mostly focused on high-risk groups such as pregnant women and immunocompromised patients[12]. However, none of the studies have been conducted to evaluate the epidemiology of *T. gondii* infection in only rural communities. In addition, Northern Iran is a highly endemic area for toxoplasmosis[12]. Considering that there are no epidemiological data showing recent prevalence of toxoplasmosis in this area, this study was designed to investigate the prevalence of *T. gondii* infection and to identify the potential risk factors associated with toxoplasmosis among rural communities in Northern Iran.

MATERIAL AND METHODS

Study area and study population

This cross sectional study was performed in Northern Iran (*Amol*, *Nor*

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and *Mahmod Abad* cities) from July 2014 to March 2015. Until recently, these cities were part of a single county called *Amol*. This area is located in a region of mountains and forests in Northern Iran. The main economic activities in this area are agriculture and animal husbandry. According to the Statistical Centre of Iran (SCI), the number of rural inhabitants in this area is about 269,000[13]. This area (36°25'N 52°21'E) has an altitude of 76 meters above the sea level, a hot summer characterized by a Mediterranean climate, a mean annual temperature of 15.9 °C and about 829 mm of rain fall annually. We selected 13 rural villages in four different counties of the cities depending on the land area and number of inhabitants. A list of all the villages from the local governor was used to select 13 villages by means of a simple random sampling technique. The participants were selected among those who referred to health centers to undergo medical examination in the primary clinical care. Selected villages were: two from the *Nor* county (65 people), three from the *Mahmod Abad* county (128 people), four villages from the *Dabodasht satae* (*Amol* county, 219 people) and four villages from the *Litkoh* & *Larijan* community (*Amol* county, 208 people). Inclusion criteria to enroll rural people were: 1) belong to the *Mazanderani* ethnicity; 2) be a resident of the rural area in the past 10 years; 3) to be > 1 year old; 4) to consent to participate in this study. Participants were excluded from the study if they did not fulfill the inclusion criteria. Moreover, pregnant women and also individuals with a history of impaired immune response were excluded from this study.

Sample size and sampling method

To calculate the sample size we used a seroprevalence of reference of 59%[12] at a confidence level of 99% and a marginal error of 0.05. The calculation ($N = \frac{Z^2 P(1-P)}{d^2}$) of the sample size for the cross-sectional study was adopted[14]. The result of the calculation was 638 subjects, and 630 participants were enrolled in the study.

Ethical aspects

This study received the approval from the local health authorities and from the *Shahid Beheshti* University of Medical Science Ethical Committee. All the enrolled participants were informed about the study and a written informed consent was obtained. In the case of minors, written consents were signed by the parents or legal guardians.

Questionnaire survey

A previously published questionnaire was used to assess the risk factors, with some modifications, mainly regarding eating habits and the type of meat consumed, such as pork meat and salami that were removed from the questionnaire[15]. Briefly, the following variables were included to measure demographics and potential risk factors: age; sex; place of residence, educational level (illiterate, primary education, high school, college graduate education and higher); occupation; soil-related activities including gardening, washing fruits or vegetables; exposure to cats and cat feces; consumption of raw or undercooked meat; type of meat consumed (sheep, beef, goat, poultry); and sources of drinking water (well, unfiltered, spring water, filtered) as shown in Table 1.

Laboratory tests

About 5 mL of whole blood samples were drawn from each participant through venipuncture. Blood samples were allowed to clot and centrifuged at 1000 g for 5 minutes. Sera were stored at 4 °C for 2-3 days until they were transported in ice to the Parasitology Laboratory of the *Shahid Beheshti* Univesity of Medical Science where they were kept at -20 °C until the analysis.

Serum samples were screened for anti-*Toxoplasma* IgG and IgM antibodies using two commercial enzyme-linked immunosorbent assays (ELISA) kits (ACON, San Diego, CA, USA) as instructed by the manufacturers. These kits have sensitivities and specificities of 99.9% and 99%, respectively. According to the manufacturer's recommendation, results were reported in International Units (IU). Sera with values of < 9.0 IU/mL were considered negative; those with values between 9.0-11.0 IU/mL were considered suspicious (gray zone), and those with values > 11.0 IU/mL were considered positive, for both *T. gondii* immunoglobulin G and M antibodies.

Table 1
Seroprevalence of *Toxoplasma* infection among rural individuals in Northern Iran according to sociodemographic characteristics (risk factors analysis by the Pearson's chi-square test)

Characteristic	No. participants	No. of seropositives (%)	OR (95% CI)	*p* value
Age (years)				<0.001
≤10	18	8 (44.4)	1.0	
11-20	60	36 (60.0)	1.88 (0.65-5.43)	
21-30	141	101 (71.6)	3.16 (1.16-8.57) **	
31-40	124	91 (73.4)	3.45 (1.25- 9.48) **	
41-50	112	93 (83.0)	6.12 (2.14-17.53) **	
51-60	109	93 (85.3)	7.27 (2.49-21.19) **	
≥61	66	55 (83.3)	6.25 (2.01-19.40) **	
Sex				0.512
Male	203	157 (77.3)	1.14 (0.77 -1.69)	

Table 1
Seroprevalence of *Toxoplasma* infection among rural individuals in Northern Iran according to sociodemographic characteristics (risk factors analysis by the Pearson's chi-square test) (cont.)

Characteristic	No. participants	No. of seropositives (%)	OR (95% CI)	*p* value
Female	427	320 (74.9)	0.88 (0.59- 1.30)	
Educational level				<0.001
Illiterate	158	132 (83.5)	1.0	
Primary school	143	118 (82.5)	0.93 (0.51-1.70)	
High school	234	166 (70.9)	0.48 (0.29 -0.80) **	
College and higher	95	61 (64.2)	0.35 (0.20-0.64) **	
Occupation				0.010
Farmer & shepherd	57	46 (80.7)	1.0	
Housewife	312	251 (80.4)	0.98 (0.48 -2.01)	
Student	99	70 (70.7)	0.58 (0.26 -1.27)	
Government employee & other	162	110 (67.9)	0.51 (0.24 -1.06)	
Region				0.992
Dabodasht	219	167 (76.3)	1.0	
Litkoh & Larijan	208	157 (75.5)	0.96 (0.62 -1.49)	
Dashtesar	138	104 (75.4)	0.95 (0.58-1.57)	
Larijan	65	49 (75.4)	0.95 (0.50 -1.81)	
Water supply				0.009
Treated	272	192 (70.6)	0.61 (0.43 -0.89) **	
Untreated	358	285 (79.6)	1.63 (1.13 -2.35) **	
Consumption of undercooked or raw meat				<0.001
Yes	386	318 (82.4)	2.50 (1.72 -3.62) **	
No	244	159 (65.2)	0.40 (0.28-0.58) **	
Consumption of raw unwashed vegetables or fruits				<0.001
Yes	322	271 (84.2)	2.63 (1.80 -3.85) **	
No	308	206 (66.9)	0.38 (0.26 -0.56) **	
Contact with cats				0.182
Yes	226	178 (78.8)	1.30 (0.88 -1.92)	
No	404	299 (74.0)	0.77 (0.52 -1.13)	
Contact with soil				0.005
Yes	317	255 (80.4)	1.69 (1.17 -2.44) **	
No	313	222 (70.9)	0.59 (0.41 -0.86) **	
Consumption of goat meat				0.655
Yes	140	108 (77.1)	1.11 (0.71 -1.73)	
No	490	369 (75.3)	0.90 (0.58 -1.41)	
Consumption of sheep meat				0.015
Yes	540	418 (77.4)	1.80 (1.11 -2.91) **	
No	90	59 (65.5)	0.55 (0.34 -0.90) **	
Consumption of cow meat				0.663
Yes	429	327 (76.2)	1.09 (0.74 -1.61)	
No	201	150 (74.6)	0.92 (0.62- 1.35)	
Consumption of poultry meat				<0.001
Yes	564	438 (77.7)	2.41 (1.42 -4.09) **	
No	66	39 (59.1)	0.42 (0.24 -0.71) **	

**95% confidence interval does not include 1.

Statistical analysis

Results were analyzed using the SPSS Statistics software, version 21 (IBM, NY, USA). The mean ± standard deviation was used to describe the continuous variables such as age and frequency and percentages were used for ordinal and nominal variables. The Pearson's chi-square (χ^2) test was used to determine the association between seropositivity for *T. gondii* infection and the risk factors. A multivariate logistic analysis was performed using the Hosmer & Lemeshow selection model[16], and odds ratios (OR) and 95% confidence intervals (CI) were calculate to associate the potential variables. A p value of < 0.05 was considered statistically significant.

RESULTS

Distribution of participants

Among the 630 subjects of the study, 67.8% (427/630) were females and 32.2% (203/630) were males. They were 3 - 78 years old with a mean age of 36.6 years (± SD 16.3). Most of the participants (69%) had middle socioeconomic status and were aged 21-40 years (42%). The majority of women (73%) were housewives. Concerning the level of education, 158 (25%) participants were unable to read and write. Socio-demographic characteristics are presented in Table 1.

Seroprevalence of anti-*T. gondii* antibodies and univariate analysis of risk factors

Among the 630 participants, 477 were seropositive for anti-*T. gondii* antibodies (75.7%, CI 95%: 72.4-79.1). Of these, 465 cases (73.8%) tested positive for only IgG, suggesting a chronic toxoplasmosis profile. Twelve cases (1.9%) tested positive for both IgG and IgM, suggesting recent toxoplasmosis. Results of the univariate analysis showed that *T. gondii* seropositivity was associated with age ($p < 0.001$), educational level ($p < 0.001$), occupation ($p = 0.01$), water supply ($p = 0.009$), contact with soil ($p = 0.005$), consumption of undercooked meat ($p < 0.001$), unwashed raw vegetables or fruits ($p < 0.001$), sheep meat ($p = 0.015$) and poultry meat ($p < 0.001$). Complete data are presented in Table 1.

Logistic regression analysis for risk factors of seropositivity to *T. gondii*

In the logistic regression analysis, four variables were identified as being associated with *T. gondii* infection: age, occupation, consumption of undercooked meat, and of unwashed raw vegetables or fruits (Table 2). As shown in Figure 1, an increasing seroprevalence with age was observed in this study ($p = < 0.001$).

DISCUSSION

In this cross-sectional study, we found a seroprevalence of 75.7% to *Toxoplasma gondii* in rural communities of Northern Iran that is significantly higher than the mean (39%) seroprevalence of *T. gondii* infection previously reported in the general population in Iran[12]. In addition, the seroprevalence in our study is higher than the 23.8% of seroprevalence to *T. gondii* infection reported in a similar Mexican population and also the 6.2% of seroprevalence found in *Kyrgyzstan*, a central Asia country[17,18]. Our prevalence rate is also much higher than those reported in the general population from Qatar (29.8%), Pakistan (29.48%) and Turkey (31.9%) in neighboring Iran[19-21]. One possible factor that may explain the high seroprevalence of toxoplasmosis in the studied rural population is the geographical climate. Climate in Northern Iran has high humidity due to the proximity to the Caspian Sea which provides optimal conditions for the sporulation and survival of *Toxoplasma* oocysts in the environment[12]. The influence of climate conditions on the oocyst survival and *T. gondii* transmission has been reported by previous studies in the world and Iran[3,12]. Another contributing factor could be due the prosperity of agriculture and animal husbandry in Northern Iran. Toxoplasmosis is one of the most prevalent zoonotic parasitic diseases, therefore more intense contact with animals or livestock increases the risk of infection[22]. Another possibility of transmission of toxoplasmosis to humans would be through contaminated environment, contaminated unwashed vegetables and consumption of undercooked meat.

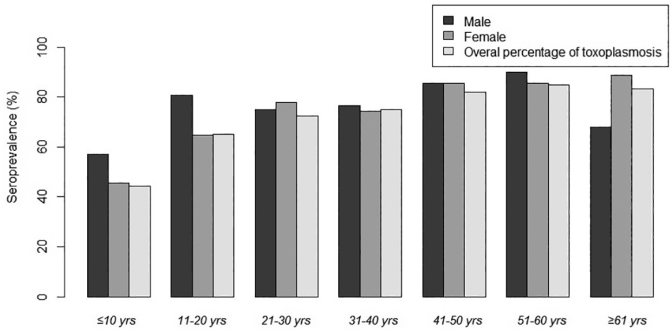

Fig. 1 - Age-associated prevalence of *Toxoplasma gondii* infections among rural populations in north of Iran.

In the present study several variables were found to be associated with *T. gondii* infection by univariate analysis (Table 1). However, in the multivariate logistic regression analysis, only four variables were identified as potential risk factors for acquisition of infection: age, occupation, consumption of undercooked meat and of unwashed raw vegetables or fruits (Table 2). These results are in agreement with previous studies from other countries[3,11,12]. The present study found that housewives and farmers are two groups with high *T. gondii* seroposivity. This finding is in agreement with previous studies performed in Iran and other areas in the world such as the United States and Saudi Arabia[12,15,23]. These high-risk groups are more exposed to potential parasite sources: pet caregivers, people who work with livestock, chop meat and clean vegetables without wearing gloves and have contact with contaminated soil during gardening[12]. Agreeing with these facts, our results demonstrated that seroposivity was significantly higher in individuals who have contact with soil in comparison with those who did not. We found that the frequency of seropositive cases increased with age (Fig. 1). Further analysis revealed that older age groups (≥ 50 years) were three times more likely to have *T. gondii* seroposive results than the younger age groups (≤ 24 years). This finding is consistent with the majority of studies in the world[12,17,22,24]. The explanation would be an enhanced exposure to toxoplasmosis by means of consumption of raw or undercooked infected products. Another possible is that older people may have had greater exposure when they were younger and this could inflate the increase in age as a risk. It is important to remember that anti-*T. gondii* antibodies persist for a long time, and the increment of the seroprevalence with age is associated with life-time exposure. Moreover, our results revealed that increasing the level

Table 2
Seroprevalence of *Toxoplasma* infection among rural individuals in Northern Iran according to sociodemographic characteristics (estimated by a full logistic regression model)

Characteristic	No. of participants	OR	95% CI	*p* value
Age (years)				
≤10	18	1.00*	1.0	
11-20	60	3.04	(0.90- 10.23)	0.73
21-30	141	11.35**	(3.19- 40.34)	<0.001
31-40	124	13.31**	(3.62- 48.97)	<0.001
41-50	112	14.04**	(4.02- 49.00)	<0.001
51-60	109	17.80**	(4.89- 64.76)	<0.001
≥61	66	11.71**	(2.86- 48.00)	<0.001
Educational level				
Illiterate	158	1.00*	1.0	
Primary school	143	1.25	(0.57- 2.78)	0.577
High school	234	0.67	(0.30- 1.49)	0.326
College and higher	95	0.44	(0.16- 1.17)	0,099
Occupation				
Farmer & shepherd	57	1.00*	1.0	
Housewife	312	1.51	(0.65- 3.49)	0.337
Student	99	3.28**	(1.13- 9.53)	0.029
Government employee & other	162	1.05	(0.42- 2.62)	0.915
Water supply				0.497
Treated	272	1.17	(0.75- 1.81)	
Untreated	358	1.00*		
Consumption of undercooked or raw meat				0.004
Yes	386	0.52**	(0.33- 0.81)	
No	244	1.00*		
Consumption of raw unwashed vegetables or fruits				0.005
Yes	322	0.53**	(0.34- 0.82)	
No	308	1.00*		
Contact with soil				0.119
Yes	317	0.72	(0.48- 1.09)	
No	313	1.00*		
Consumption of sheep meat				0.218
Yes	540 (84)	0.71	(0.42- 1.22)	
No	90 (16)	1.00*		
Consumption of poultry meat				0.889
Yes	564 (89.5)	0.96	(0.51- 1.80)	
No	66 (10.4)	1.00*		

* Reference. ** 95% confidence interval does not include 1.

of education is related with reduced rate of *T. gondii* seroposivity. Lower levels of education are associated with a lack of awareness of the disease, lower socioeconomic status, lower hygiene status and professional activity with greater exposure to contaminated soil[12,15]. The results of the present study and those of others previously published on toxoplasmosis have indicated that contaminated water could be a source of *T. gondii* infections in outbreaks[22,25,26]. Consistent with these studies, our finding showed that using untreated water (well, spring and unfiltered water) is an important risk factor to acquisition of infection. Northern Iran has a rainy climate and abundant springs, therefore the majority of villages use these springs as a source of drinking water.

In our study we observed a significant association of seropositivity with the consumption of undercooked meat and unwashed raw vegetables. The latter are one of the main sources of parasitic infections in Northern Iran and around the world[27,28]. Several studies claimed that consumption of undercooked meat is an important factor for parasite transmission[12,15,17,24,29]. However, some other studies reported that there was no association between *T. gondii* infection and consumption of meat[17,30]. Cow, sheep, goat and chicken meat are used in almost all of the Iranian typical dishes. The consumption of poultry (turkey, chicken, geese, duck) and sheep meat was related to *T. gondii* seroposivity by the univariate analysis in our study. Meat is one of the major components of the Persian cuisine and is consumed in different forms. One of the most common methods of cooking meat is in the form of different *Khoreshts* (stews) such as *Ghormeh sabzi* and *Gheimeh*. In these forms, meat is well cooked and the risk of transmitting *T. gondii* is very unlikely. However, special raw meat dishes such as *Kebab*, *Shishlik* and *Joje Kebab* are also very popular in Iran and are consumed lightly cooked. To prepare *kebab*, the preferred meat is lamb, but other meats such as beef, goat and poultry are also used. *Kebab* and other crude meat are known festive recipes, and are served in most restaurants. These undercooked meat dishes served in most guests, and every Iranian eats it almost once a week. Previous studies revealed that seroprevalence of *T. gondii* infection among cow, goat and sheep in Northern Iran was 9%, 14.2-30% and 20-90%, respectively[31,32].

Contact with cats was not associated with *T. gondii* seropositivity in the present study. Similar results were observed by Alvarado-Esquivel *et al.* in a study conducted in a rural Mexican population, and also by Walle *et al.* in a study in Ethiopia[17,33]. A possible explanation for this finding could be attributed to religious beliefs of the inhabitants in the studied areas, as they do not have a close contact with cats.

Our study had some limitations. It would have been ideal to include more rural communities and more participants but this was not feasible due to the time to finish the study and also financial constraints. Another limitation of our study is the risk factor analysis based on IgG antibody titers, considering that seropositivity for *T. gondii* persists for many years and positive individuals were likely to have been infected a long time ago, and their living conditions and behaviors may have changed over time. Therefore a direct temporal relationship between behaviors and *T. gondii* infection could not be established.

In conclusion, our results demonstrate a high seroprevalence of *T. gondii* infection in rural populations of Northern Iran. Age, consumption of undercooked meat and raw vegetables and occupation are important risk factors to acquire *T. gondii* infection in rural areas. A health program should be established to increase to increase people's knowledge regarding toxoplasmosis, the identified risk factors, and the negative impact on the public health that the lack of governmental measures would cause.

CONFLICT INTEREST

None.

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AUTHORS' CONTRIBUTION

A. Rostami and S.J. Seyyedtabaee designed the study, performed the research, evaluated the clinical records and collaborated to the manuscript writing. S. Aghamolaie, H. Behniafar and Z. Lasjerdi collaborated in the sample collection and to perform the laboratory tests. S. Mehravar performed the statistical analysis and has analyzed the data. A. Abdolrasouli and C. Alvarado-Esquivel have critically reviewed the manuscript.

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