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Sero-Epidemiology of Toxoplasmosis among the People of Khorram Abad, Iran

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Abstract

Toxoplasma gondii is an opportunistic, zoonotic pathogen with a worldwide distribution. There are large variations in the seroprevalence of Toxoplasma infection in different regions of the world. Although toxoplasmosis became a notifiable sectional study aimed to survey the seroprevalence of Toxoplasma infection and its risk factors among people in Khorram Abad. In the present study, simple random sampling was carried out on the referents to 6 medical diagnostic laboratories (from among the 9 laboratories available in the city). The tests were performed on the 1000 blood serum samples using the IFA (immunofluorescent antibody assay) method and the effects of three variables including place of residence, occupation, and marital status were considered. Of the 1000 Khorram Abad studied, 831 (83.1%) were positive for Anti-*T. gondii* IgG antibody and 169 (16.9%) were negative. Seroprevalence of Toxoplasma infection did vary with residency, material status, and occupation ($P<0.05$). Our results demonstrate serological evidence of Toxoplasma exposure among Khorram Abad which may be impacting their health. Results of this first study of Toxoplasma infection in Khorram Abad may be useful for the design of optimal preventive measures against infection with Toxoplasma.

Keywords: Toxoplasma gondii; Seroprevalence; Khorram Abad; Cross-sectional study

Introduction

The ultimate host for the parasite *Toxoplasma gondii* (*T. gondii*) is the felines, which excrete the parasite in the environment, and then the parasite enters human body and other animals by different paths [1-3].

There are usually no symptoms indicating infection with *Toxoplasma gondii*, but first-time infection during pregnancy leads to miscarriage or congenital defects. In individuals who are affected by compromised immune systems, this parasite can be considered as an opportunistic pathogen [4-6]. Application of the vaccine (dead parasites) among laboratory animals has produced mixed results of varying degrees of success. In susceptible animals (e.g. mice and rabbits) vaccination has not led to immunity, while vaccinated guinea pigs have resisted a deadly repeat dose of pathogenic *T. gondii*. Up to now, no effective vaccine has been produced for humans, since the dangers of using a live vaccine in humans is quite clear, and if dead and inactive parasites are used, many problems will arise in immune response to such a vaccine. To treat this disease, sulfadiazine (or sulfamerazine, or sulfamathazine) together with pyrimethamine (Daraprim) are used. These drug combinations are effective against *T. gondii* at the trophozoite stage, yet have no effect on cysts. As a result, treatment may change the mode (behavior) of the acute stage of the disease, but cannot prevent the incidence of chorioretinitis [7-12].

Toxoplasmosis is of cosmopolitan distribution and studies of antibodies have shown that between 20-75 percent of different populations are chronically but asymptomatically infected with this parasite. Although most infection with *T. gondii* is asyptomatic, some infected individual may suffer from symptomatic pathological changes in the lymph nodes, eye, and central nervous system [13]. In addition, pregnant women with primary infection with *T. gondii* may transmit the infection with to the fetus leading to congenital disease [14,15]. In areas where cats are numerous, sanitation and health services are inadequate, humidity is high and temperatures are moderate, there can be found antibodies against *T. gondii* in a large proportion of children. The same is true for areas where meat is eaten raw or rare [11].

Due to the importance of this protozoan zoonosis and the lack of data about its prevalence in our area, we conducted a seroepidemiological study to assess the prevalence of human toxoplasmosis and the epidemiological factors associated with the spread of the disease

Materials and Methods

Study design and study population

In this study, samples were taken from the referents to 6 Medical Diagnostic Laboratories from among the 9 laboratories available in the Khorram Abad city during January to December 2013.

Serological examination for *T. gondii* antibodies

The tests performed on the blood serum samples. In brief, serum concentrations are reduced to the following degrees using PBS (phosphate-buffered saline) including 1:20, 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, and 1:6400. Drops of the different concentrations of the serum are placed on the antigen smears, which have been fixated on the slide. A positive serum and a negative serum are used as the control sample. Then, the slides are placed inside a humid chamber in an incubator at 37°C, so that the specific antibody present in the serum

reacts with the antigen. At the next phase, the slides are taken out of the humid chamber and put in a wash bottle (Chamber) containing PBS. The slides are washed twice, and each time they are left in PBS for ten minutes. During this phase non-specific antibodies are removed. After washing, the slides are set in a slanted angle for the surface liquid to be removed. Then 5 ml of the diluted conjugated serum, to which 5 µl Evans Blue with a concentration of 1/10000 was added, is placed on antigen smears, and the slides are put in the humid chamber in the incubator at a temperature of 37°C for 30 min.

In this part of the study, the conjugated antihuman globulin is attached to the combination of the antibody and the antigen and causes the parasite does not glow under the immunofluorescent microscope. If no antibody is present, the conjugated antihuman globulin is washed during the rinsing process and the parasite does not glow under the microscope. Next, the rinsing of the slides with PBS is repeated as before. Then, a few drops of diluted glycerin are placed on the slides and the slides are covered with coverslips. The outcome can be examined using an immunofluorescence microscope with a magnification of 50.

Statistical analysis

The statistical analysis was performed using SPSS18.0 software package (IBM, Armonk, New York, USA). P values less than 0.05 were considered statistically significant. Logistic regression was used to analyze the association between *T. gondii* seropositivity and potential risk factors. Multivariate logistic analysis was performed with the full model, including all potential risk factors in the analyses.

Results

Of the 1000 Khorram Abad studied, 831 (83.1%) were positive for Anti-*T. gondii* antibody and 169 (16.9%) were negative. After performing the tests on the serums using the above-mentioned methods and cross-matching the findings of the tests with the three factors of place of residence, occupation, and marital status, the following results were obtained from table 1, it can be seen that 78 percent of the samples belonged to urban residents and 22 percent belonged to rural residents. Using the likelihood ratio test, it was obvious that there is a significant difference between the two groups of urban and rural residents (P<0.05) (Table 1).

Place of residence	Positive (n, %)	Negative (n, %)	Sum (n, %)
Urban areas	122, 15.6	658, 84.4	780, 100
Rural areas	47, 21.4	173, 78.6	220, 100
Sum	169, 16.9	831, 83.1	1000, 100

Table 1: Distribution of absolute and relative frequencies of the anti- *T. gondii* antibody assays based on the place of residence

Infection rates are higher in rural areas than in urban areas, despite the fact the number of referents from urban areas is higher. From the data, it can be concluded that the highest referral rates based on occupation belongs to housewives (32.2%) and the lowest to hospital staff (4.3%). Statistically, the chi-square test indicated that the highest infection rates belonged to farmers and laborers (Table 2). Table 3 indicates that 81.9 percent of the subjects were married and 18.1 percent were single. The likelihood ratio test showed that infection rates were higher among single subjects than among married subjects (P<0.05).

	Male			Female			Sum		
Occupation	Infected	Non-infected	Total	Infected	Non-infected	Total	Infected	Non-infected	Total
	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)	(n, %)
Teachers & office employees	10,13.5	64,86.5	74,100	8,15.6	43, 84.3	51,100	18,14.4	107,85.6	125,100
Craftsmen	6,17.6	28,82.4	34,100	6,40	9, 60	15,100	12,24.5	37,75.5	49,100
Hospital staff	3,10.7	25,89.3	28,100	0, .00	15,100	15,100	3,7.0	40,93.0	43,100
Farmers & laborers	37,64.9	20,35	57,100	16,19.0	68,80.9	84,100	53,37.6	88,62.4	141,100
Housewives	0,0.0	0,0.0	0,0.0	54,16.8	268,83.2	322,100	54,16.8	268,83.2	322,100
School & university students	7,14.6	41,85.4	48,100	8,28.6	20,71.4	28,100	15,19.7	61,80.3	76,100
Other occupations	14,25.0	42,75.0	56,100	0,0.0	8,100	8,100	14,21.9	50,78.1	64,100
Sum	77,17.3	367,82.7	444,100	92,16.5	464,83.5	556,100	169,16.9	831,83.1	1000,100

Table 2: Absolute and relative frequency distributions of anti- *T. gondii* antibody assays based on occupation and sex

Antibody assay results Marital status	Positive (n, %)	Negative (n, %)	Sum (n, %)
Married	127, 15.5	692, 84.5	819, 100
Single	42, 23.2	139, 76.8	181, 100
Sum	169, 16.9	831, 83.1	1000, 100

Table 3: Absolute and relative frequency distribution of anti-*T. gondii* antibody assays based on marital status

Discussion

The present study was performed to investigate the seroepidemiology of Toxoplasma infection in Khorram Abad in Iran. Results indicate that 78% of the subjects lived in the urban areas and 22% lived in rural areas. Despite the larger number of referrals from city dwellers, residents of rural areas are statistically more likely to be infected with the parasite. A main finding of the study was significant difference ($P<0.05$) between seroprevalence in the rural population compared with the urban population to *T. gondii*. This is consistent with report that suggesting that population from rural areas is highly exposed to the parasite.

Toxoplasmosis is seen more frequently among farmers and laborers due to more frequent contact and consumption of contaminated food and poor hygiene. According to the occupation, the highest referral rates belong to housewives (32.2%) and the lowest to hospital staff (4.3%), and statistically speaking the highest infection rates were observed among farmers and laborers ($P<0.05$).

We did find a significant correlation between marital statuses with *T. gondii* seroprevalence. According to this, 81.9 percent of the subjects were married and the rest were single. Statistically (based on the likelihood ratio test ($P<0.05$), single participants were more likely to be infected than married participants.

In a study by Sun et al. conducted on 880 clinically healthy individuals, the overall prevalence among the studied sample was 20 percent [10]. In another study by Mwambe et al., it was reported that, of the 350 pregnant women under study in Tanzania, a total of 108 (30.9%) were positive for anti-*T. gondii* antibodies [11]. A post-mortem study on Polish subjects reported by Samojlowicz et al., reported that 55 percent of the 169 people studied had been infected with *T. gondii* [12].

In conclusion, our results demonstrate serological evidence of *T. gondii* exposure in Khorram Abad may be impacting their health. This is the first report of Toxoplasma infection in Khorram Abad, and results should be useful for the optimal design of preventive measures against *T. gondii* infection.

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