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# Prevalence of human toxoplasmosis and related risk factors using Electrochemiluminescence (ECLIA) method in West Azarbaijan Province, Iran, 2010

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**Abstract**

Toxoplasmosis is a cosmopolitan protozoan infection caused by *Toxoplasma gondii*. Cats and other felids are its definitive host and infect most genera of vertebrates including humans as well as cat as intermediate hosts. The aim of this seroepidemiological study was to determine the frequency of infection in West Azarbaijan province, Iran, during 2011 using Electrochemiluminescence method. In this cross sectional study 200 blood samples were collected randomly from human cases reffered to the Urmia City main laboratories from almost all cities of West Azerbaijan province and also demographic variables of the subjects were collected by interviewing. Anti-*Toxoplasma* IgM and IgG concentrations were determined in samples using Electrochemiluminescence method. Ninety six (47%) out of 200 samples showed the positive level of anti-*Toxoplasma* IgG, however, 7 samples (3.5%) were positive for IgM. Nine samples (4.5%) showed the IgG concentration level in borderline. Only in one sample IgM was positive alone and therefore, among the participants of this study, 6 people had acute and 80 people had chronic infection. Significant statistical relationship between frequency of toxoplasmosis with occupation and the method of washing everyday using vegetables were observed. There was no statistically significant relationship between the frequency of *Toxoplasma* infection with sex, age, educational and marital status, residential status, cat and soil contact, eating undercooked meat and history of hospitalization. This study indicated the prevalence of *Toxoplasma* infection is high in the residents of West Azarbaijan Province, Iran. However, relatively high acute infection rate in this population could not be ignored.

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## Introduction

*Toxoplasma gondii* is an apicomplexan intracellular protozoon parasite, which has a broad range of intermediate hosts, infects about all warm-blooded animals (mammals and birds) including humans (Nardoni *et al.*, 2011; Tenter *et al.*, 2000). Interestingly cat itself can be an intermediate host. This animal is responsible for contaminating the environment. The infection to *Toxoplasma* occurs when intermediate hosts such as humans eat food or drink water containing the sporulated oocysts. Also, eating undercooked meat containing bradyzoites is another way of acquiring the infection (Putignani *et al.*, 2011). Oocysts are mostly resistant to different environmental factors, and have the ability to remain infective for a long period of time in soil as well as on other soil contaminated fruits and vegetables. In addition, the infective dose of oocysts is fairly low (Dubey, 2004; Nardoni *et al.*, 2011). *Toxoplpsma* infection was underestimated before aids become epidemic, but it illustrated as a considerable clinical problem globally. In 1948 Sabin and Feldman coined the dye test for diagnosis of *Toxoplasma* infection (Sukthana, 2006). Their method nowadays is using as a gold standard in serological tests for diagnosis of toxoplasmosis (Reiter-Owona *et al.*, 1999). The infection is mostly manifesting no symptom in immunocompetent individuals, but in the condition of immunodeficiency, it causes severe diseases such as toxoplasmic encephalitis. Early diagnosis of acute infections are vital, especially in pregnant women (Sukthana, 2006). In Iran epidemiological studies shows different prevalency rates of infection in different studied regions. The infection rate in whole population of the country is estimated 51.8% (Assmar *et al.*, 1997). For establishing better health policies, it is necessary to monitor the infection rate and have updated information about the prevalence and risk factors associated with the infection. We performed a cross-sectional study to determine the prevalence of *T. gondii* infection in West Azarbaijan province, on the people who referred to the Urmia city laboratories from about whole Province using Electrochemiluminescence method.

## Materials and methods

### Sampling

In this cross sectional study 200 serum samples were collected from subjects referred to the Urmia city diagnostic laboratories from almost all cities of West Azarbaijan province including; Urmia, Noushinshahr, Khoy, Miandoab, Maku, Mahabad, Piranshahr, Salmas, Shahindej, Bukan, Sardasht, Nagadeh, Oshnaviye, Takab and Poldasht during 2010. Sample size calculated using the prevalence of the infection from neighboring regions(Jafari *et al.*, 2012). Data were collected about the demographic variables of subjects, such as; age, sex, marital status, education, occupation, history of hospitalization, blood transfusion, keeping cat at home or roaming of stray cats near their dwelling, having contact with soil, eating raw vegetables, how they wash the vegetables and consumption of under cooked livestock or game meat. Blood samples from the participants of the study have been taken and the sera were kept frozen at -20°C until the examination.

### Enzyme Linked Immunosorbent Assay

After the sampling was ended, all the sera thawed at room temperature to determine anti-*Toxoplasma* IgG and IgM. Electrochemiluminescence immunoassay kits (Cobas® England) applied in Roche Elecsys 2010 device (Hitachi, Ltd) to determinate anti-*Toxoplasma* IgM and IgG titers in the sera according to the manufacturer's instruction. IgG level under 1 IU/ml considered negative, 1-3 IU/ml undetermined and higher than 3 IU/ml considered positive, also IgM level under 0.9 IU/ml regarded as negative, 0.9-1.1 IU/ml undetermined and higher than 1.1 IU/ml considered being positive as the company suggestion.

### Data analysis

Data analyzed by SPSS v.16 software using Chi-Square test and *P*<0.05 regarded as significant.

## Results

### Prevalence of the infection

A total of 94 samples (47%) out of 200 were IgG positive, 9 (4.5%) were borderline and also 7 (3.5%) were IgM positive. Totally 88 (44%) among all

studied population had the chronic toxoplasmosis (IgM⁻ , IgG⁺) and 6 people had acute or subacute infection (IgM⁺ , IgG⁺). Just one (0.5%) out of 200 people was IgM positive alone.

*Infection and possible risk factors*

Frequency of IgG positivity among people from rural areas was 54.4%, however, 43.18% from urban area were positive. Accordingly, frequency of IgM positivity was 4.41% and 3.03% in the rural and urban areas, respectively. Differences in frequency of the infection in the rural and urban residents were not significant statistically (Table 1).

**Table 1.** Odds ratio estimated for anti-*Toxoplasma* IgG and IgM seropositivity among residents of rural and urban areas.

| | IgG | | Total | OR | *P* value | IgM | | Total | OR | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Positive n(%) | Negative n(%) | | | | Positive n(%) | Negative n(%) | | | |
| Rural | 37(54.4) | 31(45.6) | 68 | 1.570 | 0.132 | 3(4.41) | 65(95.9) | 68 | 1.477 | 0.445 |
| Urban | 57(43.18) | 75(56.8) | 132 | 1 | | 4(3.03) | 128(96.97) | 132 | 1 | |
| Total | 94(47) | 106(53) | 200 | | | 7(3.5) | 193(96.5) | 200 | | |

Maximum frequency of IgG and IgM seropositivity were in age groups older than 40 years 62.5% and 6.25%, respectively, which was not significant statistically (Table 2).

**Table 2.** Frequency of *Toxoplasma* infection in different age groups.

| Age group | IgG | | Total | *P* value | IgM | | Total | *P* value |
|---|---|---|---|---|---|---|---|---|
| | Positive n(%) | Negative n(%) | | | Positive n(%) | Negative n(%) | | |
| <10 | 2(28.6) | 5(71.4) | 7 | 0.070 | 0(0) | 7(100) | 7 | 0.891 |
| 11-20 | 13(31.7) | 28(68.3) | 41 | | 1(2.4) | 40(97.6) | 41 | |
| 21-30 | 42(47.2) | 47(52.8) | 89 | | 3(3.4) | 86(96.6) | 89 | |
| 31-40 | 17(54.8) | 14(55.2) | 31 | | 1(3.2) | 30(96.8) | 31 | |
| >40 | 20(62.5) | 12(37.5) | 32 | | 2(6.2) | 30(93.8) | 32 | |
| Total | 94(47) | 106(53) | 200 | | 7(3.5) | 193(96.5) | 200 | |

IgG titers were positive in 38.96% of males and 52.03% of females. Also IgM titers were positive in 3.89% of males and 3.25% of females. In this study, IgG seropositivity in married and single group was 45.7% and 48.38%, respectively. Accordingly, 4.67% of married and 2.15% of single participants were IgM seropositive. There was no statistical significant difference about the infection rate among the genders.

**Table 3.** Distribution of *Toxoplasma* infection in different educational groups.

| Education | IgG | | Total | *P* value | IgM | | Total | *P* value |
|---|---|---|---|---|---|---|---|---|
| | Positive n(%) | Negative n(%) | | | Positive n(%) | Negative n(%) | | |
| Illiterate | 10(71.4) | 42(28.6) | 14 | 0.069 | 1(7.1) | 13(92.9) | 14 | 0.542 |
| Elementary | 43(50.6) | 42(49.4) | 85 | | 4(4.7) | 81(95.3) | 85 | |
| High school | 31(44.9) | 38(55.1) | 69 | | 2(2.9) | 67(97.1) | 69 | |
| University | 10(31.2) | 22(68.8) | 32 | | 0(0) | 32(100) | 32 | |
| Total | 94 | 106 | 200 | | 7 | 193 | 200 | |

The highest frequency of IgG and IgM seropositivity were in illiterate educational group 71.4% and 7.1%, respectively, which was not significant statistically different comparing to other educational groups (Table 3). This study also showed the highest frequency of IgG and IgM seropositivity in housewives and farmers, whereas the lowest frequencies were seen in office staff and students (Table 4).

**Table 4.** Distribution of *Toxoplasma* infection among different occupational groups.

| Job | IgG | | Total | *P* value | IgM | | Total | *P* value |
|---|---|---|---|---|---|---|---|---|
| | Positive n(%) | Negative n(%) | | | Positive n(%) | Negative n(%) | | |
| Housewife | 36(62.1) | 22(37.9) | 58 | 0.029 | 3(5.2) | 55(94.8) | 58 | 0.461 |
| Office staff | 12(32.4) | 25(67.6) | 37 | | 1(2.7) | 36(97.3) | 37 | |
| Worker | 14(51.9) | 13(48.1) | 27 | | 2(7.4) | 25(92.6) | 27 | |
| Student | 16(34) | 31(66) | 47 | | 0(0) | 47(100) | 47 | |
| Self-employee | 8(47.1) | 9(52.9) | 17 | | 0(0) | 17(100) | 17 | |
| Farmer | 8(57.1) | 6(52.9) | 14 | | 1(7.1) | 13(92.9) | 14 | |
| Total | 94(47) | 106(53) | 200 | | 7(3.5) | 193(96.5) | 200 | |

Sixty nine out of 123 women were married and among them 62 had history of pregnancy, from those, 35 (56.45%) and 4 (6.45%) were IgG and IgM seropositive, respectively. Although based on answered questionnaires there was no history of congenital disorders in the seropositive women's infants after birth.

Among all participants, 57 people had the history of hospitalization and blood transfusion that 56.14% and 3.5% of them were IgG and IgM seropositive, respectively. Also IgG and IgM seropositivity in peoples without hospitalization were 43.35% and 3.49%, respectively.

Based on the data gathered from questionnaires, 76 people claimed they had cat as pet or stray cats commute in or around their dwelling. Results showed that 55.26% and 5.26% of this group were IgG and IgM seropositive, respectively. Also in the group without the mentioned condition, IgG and IgM seropositivity were 41.93% and 2.41%, respectively.

Sixty three amongst studied group had soil related job that 42.2% and 4.79% of them were anti-*Toxoplasma* IgG and IgM seropositive, respectively. Although in group that had not the condition 45.98% and 2.91% were IgG and IgM seropositive, respectively (*P*=0.029).

Based on a fact that studied population mostly used fresh vegetables in their daily meals, the method of washing the raw vegetables and their IgG and IgM seropositivity were compared as in Table 5.

**Table 5.** Distribution of *Toxoplasma* infection among different methods of washing vegetables.

| Method of washing vegetables | IgG | | Total | *P* value | IgM | | Total | *P* value |
|---|---|---|---|---|---|---|---|---|
| | Positive n(%) | Negative n(%) | | | Positive n(%) | Negative n(%) | | |
| Just water | 70(66.6) | 35(33.4) | 105 | <0.001 | 4(4.8) | 101(95.2) | 105 | 0.862 |
| Disinfectants | 2(16.7) | 10(83.3) | 12 | | 0(0) | 12(100) | 12 | |
| Detergents | 11(31.4) | 24(68.6) | 35 | | 1(2.1) | 34(97.9) | 35 | |
| Disinfectant/detergent | 11(22.9) | 37(77.1) | 48 | | 1(2.1) | 47(97.9) | 48 | |
| Total | 94(47) | 106(53) | 200 | | 7(3.5) | 193(96.5) | 200 | |

Among all participants, 172 (86%) had the history of eating undercooked meat (barbecued) which 48.25% and 3.48% of them were IgG and IgM seropositive, respectively, whereas in people who didn't have the condition 39.28% and 3.57% were IgG and IgM seropositive, respectively but the difference was not significant. Additionally, among the 15 (7.5%) people who had the history of eating game meat 53.33% and 6.66% were IgG and IgM seropositive, respectively compared to those who didn't had the condition, with 46.48% and 3.24% IgG and IgM seropositivity, respectively.

**Discussion**

In overall, in 200 studied samples, the rate of anti-*Toxoplasma* IgG and IgM positivity were fairly high; 47% and 4.5%, respectively, comparing to the neighboring city (Jafari, Sadaghian, and Safari 2012). Different epidemiological studies have been conducted in some parts of the Iran, for example: Ghornabi *et al.*, in 1981 found the prevalence of toxoplasmosis 23.2% in Maku city (neighboring region) and Urmia city in northwestern Iran. Lower prevalence that they found can be caused by low sensitive methods they used at the time (Ghorbani *et al.*, 1981). Jafari *et al.*, in 2012 reported the prevalence of the infection 35.1% in Tabriz City, northwestern Iran. They investigated the risk factors for infection and found that soil related jobs and eating raw vegetables at restaurants are risk factors for infection(Jafari *et al.*, 2012). In another study in 2009 among people living in southern Tehran, the prevalence of anti-*Toxoplasma* antibody among people was 68.4% which by increasing the age, the prevalence rose. This study's results indicate the higher prevalence in comparison with ours and our finding supports those finding about higher prevalence in elderly people (Salahi-Moghaddam *et al.*, 2009). Ghorbani *et al.*, (1978) studied seroprevalence of toxoplasmosis in a rural area in northern Iran and 55.7% were infected, which is higher than our studied region (Ghorbani *et al.*, 1978). Our data shows lower prevalence of infection comparing to report of Assmar *et al.*, (1977) studied in a similar way, but among general population of

Iran, the reported prevalence is close to our findings 51.8% (Assmar *et al.*, 1997).

Because of the humid environment of Caspian sea shores in northern Iran, the prevalence rates reported from these regions is very high about 55.7% and 87% (Breugelmans *et al.*, 2004; Fallah *et al.*, 2008). Reported prevalence rates varies from 6% up to 23% infection rate in the northwestern Iran and 9.3% up to 26% infection rate in southwestern Iran (Ghorbani *et al.*, 1981; Sedaghat *et al.*, 1978). In our study despite the numerical difference in frequency of IgM and IgG seropositivity in different sexes, the difference was not statistically significant, which this findings are similar to those Jafari *et al*, reported in 2012 (Jafari *et al.*, 2012).

Fallah *et al.*(2008) found seroprevalence of *Toxoplasma* infection in primigravida women in Hamadan city 33.5%, also in their study highest frequency observed among illiterate women , which is similar to our findings, also like our findings, seropositivity in women who had history of eating undercooked meat (for example barbecued) and who consumed raw vegetables was more frequent (Fallah *et al.*, 2008).

Our findings showed increase in the frequency of infection by the age and, highest rate seen in 40 year-old and higher. This increase may be caused by rise in exposure to different infected materials during their life time. This finding is similar to other reports from Hamadan city, Kermanshah and Isfahan Province (Fallah *et al.*, 2008; Mansouri *et al.*, 2003; Mostafavi *et al.*, 2011).

In the most parts of Iran, the majority of people do not keep household cats at home, but the large population of stray cats is considerable which can be important risk factor for acquiring the infection. In this study the frequency of infection was higher in people in contact with cats, but it was not statistically significant, which is similar to some other studies such as Salahi-Moghadam *et al.*( 2009) and Jafari *et al.*, 2012 (Salahi-Moghaddam *et al.*, 2009).

Though the frequency of IgM and IgG seropositivity were higher in people who were in contact with soil (history of soil related jobs) this difference was not significant. In contrast Jafari *et al.* (2012) reported soil related jobs as a risk factor for the infection.

One of the important aspects of this study is that the residents of the region consume relatively large amount of raw vegetables daily, which is a custom among Iranian population. Totally 52.5% of studied people wash their everyday consumed vegetables, only by water. Our finding showed the highest rate of IgG and IgM seropositivity among people that wash their everyday consuming raw vegetables just by water and lowest in a group that used disinfectants which this difference was significant in IgG seropositivity ($P<0.001$), but the difference in IgM seropositivity among groups was not significant ($P=0.862$). The similar results reported by Jafari *et al.*( 2012)(Jafari *et al.*, 2012).

Among the group that had the history of hospitalization and blood transfusion, the frequency of infection was higher, but it was not significant ($P>0.05$).

## Conclusion

This study indicates that the prevalence of the infection is high in the West Azerbaijan Province, Iran. However, relatively high acute infection rate in this population is considerable. Factors such as occupation and methods of washing raw foods especially everyday consumed raw vegetables are influential to acquiring the *Toxoplasma* infection.

## Acknowledgment

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