# Seroepidemiological Study of Toxoplasmosis in Women Referred to Arak Marriage Consulting Center during 2012-2013

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### Abstract

**Background:** Toxoplasmosis is a zoonotic and usually asymptomatic infection. This study was carried out to investigate the seroepidemiology of *Toxoplasma* infection in women referred to Arak Marriage Consulting Center during 2012-2013.

**Methods:** In this cross-sectional study, serum samples were collected from 400 women referred to Marriage Consulting Center in Arak City, Markazi Province, central Iran during 2012-2013. Anti-*Toxoplasma* IgG and IgM antibodies were measured by ELISA using homemade antigen. Results were analyzed by SPSS 18 and the correlation between toxoplasmosis and some affecting factors were estimated.

**Results:** Overall, 97 cases (24.3%) had IgG antibodies against *T. gondii* and 19 cases (4.8%) were positive for IgM antibodies. A significant correlation was seen between *T. gondii* infection with clinical symptoms, keeping cat as pet animal, education and handling or eating raw or undercooked meat ($P < 0.05$).

**Conclusion:** The prevalence of (24.3%) for *Toxoplasma* infection was seen in Arak City. It seems that keeping cat as pet and consumption of undercooked liver and uncooked hamburger are the most important transmission routes for the infection in this city. Since the majority of women are *Toxoplasma* sero-negative (75.7%) in Arak City, using serological tests and health education prior to marriage or during pregnancy is recommended.

**Keywords**: *Toxoplasma* infection, Marriage, Antibody, ELISA, Iran

## Introduction

*Toxoplasma gondii* is an obligate intracellular parasite. After host cell invasion, the parasites replicate by endodiogeny, which eventually leads to lysis of the host cell and subsequent invasion of neighboring cells (1).

Toxoplasmosis is often asymptomatic (2) and regarded as an opportunistic disease in immunocompromised patients (3). Acute toxoplasmosis in pregnant women affects the unborn child. In early pregnancy damage of brain, liver, spleen and eye disorders may occur in fetus (4). The infection can cause loss of fetus or high mortality and severe neurological squeal in developing fetus if it acquires during the pregnancy (5).

The diagnosis is routinely based on serological methods with detection of specific antibodies. Different serological examinations such as ELISA, IFA, latex-agglutination, and hemagglutination tests have been used for detection of *T. gondii* infection (6). In general, IgG antibody appears two to three weeks after acute infection, peaks in six to eight weeks and often persists lifelong. Detection

of IgM antibody is a tool for diagnosis of acute infection, although it remains detectable after months or years in some cases (7).

There are some studies from different parts of the world for evaluating of *T. gondii* infection in pregnant women with different results such as 30% in Spain, 22.1% in Slovakia, 24.6% in Turkey (8-10). Moreover, toxoplasmosis has been reported amply in Iran and varied according to risk factors: age, geographic area, eating habits, pet keeping and etc (11).

Because of the lack of prevalence study of toxoplasmosis in Arak city, existing risk factors in the area and since 80-90% of infected patients are asymptomatic, it is expected that a lot of people to be at risk of *T. gondii* infection there. Due to preventive measures and strategies in congenital toxoplasmosis, estimation of population at risk of infection and its correlations with risk factors are essential. As a result, this study was performed to evaluate seroepidemiology of *T.gondii* infection in women referred to marriage consulting center in Arak City, Markazi Province.

## Materials and Methods

### Collecting samples

In this cross-sectional study, random and passive sampling was applied. Blood, 2-3 ml was taken from each woman referred to Marriage Consulting Center in Arak City Markazi Province, central Iran during 2012-2013. Sera were separated by blood centrifugation at 3000 rpm for 5 min. Serum samples were transferred to the Department of Medical Parasitology and Mycology, School of Public Heath, Tehran University of Medical Science and stored in -20°C until use. Demographic and epidemiologic characteristic forms filled for each volunteers.

### Preparation of Toxoplasma antigen

Tachyzoites of *T. gondii* (RH strain) were collected from the peritoneal cavity of mice injected 3 days earlier. Tachyzoites were washed with PBS (pH 7.2) 3 times, sonicated, and centrifuged at 12000 rpm for 1 h, and the supernatant was collected as the soluble antigen. The protein content was measured by the method of Bradford.

### ELISA

The 96 well microtiter plates (Nunc, New York, USA) were coated with 5 µg/ml of diluted protein in carbonate buffer (pH 9.6) and incubated overnight at 4°C. After 3 times of washing, skimmed milk (2.5% in PBST) was added as blocking buffer. After incubation and washing serum dilution of 1/200 in PBST was added, followed by 1 hour incubation and 3 times of washing, and then anti-human IgG conjugated with horse-radish peroxidas (HRP) (Dako, Denmark) in dilution of 1/1000 was added. After incubation and washing chromogenic substrate ortho-phenylen diamidine (OPD) (Merck, Germany) was added .The reaction was stopped by adding of sulfuric acid 10%.The optical density (OD) was recorded by an automated ELISA reader

For detection of IgM antibody, 7.5 µg/ml of soluble antigen, serum dilution of 1/20, and anti human IgM conjugated in dilution of 1/500 were used respectively (8).

### Statistical analysis

The sample size was calculated 400, according to the formula: $n = z^2{}_{1-\alpha}/2p(1-p)/d^2$ Results were shown as positive or negative in comparison with cut off. Cut off was estimated by this formula: cut off= $\overline{X}$ +2SD. Data were analyzed by SPSS software, version 18 (Chicago, IL, USA). Antibodies level (IgG, IgM) and correlations with variables such as place of residence, profession, education level, contact with pets specially cat, consumption of raw vegetables and undercooked meat and receiving blood products were evaluated.

## Results

Ninety-seven cases (24.3%) of 400 women were IgG positive. Positive cases of IgG were analyzed by IgM-ELISA and 19 (4.8%) cases were positive for IgM antibodies.

According to the study of correlation between education levels and prevalence of toxoplasmosis,

the highest frequency was seen in primary school education group (41.5%), where the lowest frequency of toxoplasmosis calculated for illiterate women (16.4%) (Fig.1). With CI=95% the correlation between education level and toxoplasmosis is significant.
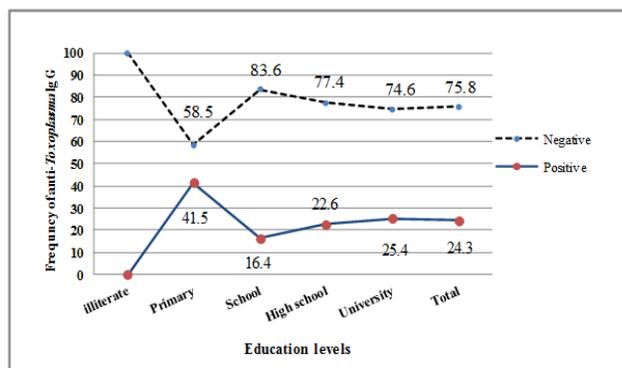


**Fig.1:** Frequency of anti-*Toxoplasma* IgG and education levels in women referred to marriage consulting center in Arak city by ELISA.

52.4% of women, which had contact with cats, had IgG antibody against *T. gondii*, whereas 20.9 % of women that had no contact with cats were infected with *Toxoplasma*. Statistical analysis showed significant correlation between contact with cats and infection rates.

82.6% of women consuming undercooked meat were infected with *Toxoplasma*, whereas 20.7% of women had anti-*Toxoplasma* antibodies without consumption of undercooked meat and meat products. As a result, undercooked meat consumption is effective in infection of women with *Toxoplasma gondii*. In addition, $P<0.05$ showed significant correlation between these two variables.

Evaluation of factors affecting toxoplasmosis in 400 individuals aged 12 to 53 years old, showed that the lowest positive cases were in the age groups of 33-38 (zero percent) and the highest in the age groups of 28-33 (27.8%) (Table 1). One-way-ANOVA and chi- square tests showed none significant differences.

**Table 1**: Frequency of anti-*Toxoplasma* IgG in age groups in women referred to marriage consulting center in Arak city by ELIS

| Age groups (yr) | Positive n (%) | Negative n (%) | Total n (%) |
|---|---|---|---|
| 12-18 | 18 20.5) | 70 (97.5) | 88 ( 22) |
| 18-23 | 48 (27) | 130 (73) | 178 ( 44.5) |
| 23-28 | 23 (23.5) | 75 (76.5) | 98 (24.5) |
| 28-33 | 5.0 (27.8) | 13 (72.2) | 18 (4.5) |
| 33-38 | 0.0 (0) | 5.0 (100) | 5.0 (1.3) |
| 38-53 | 3 (23.1) | 10 (76.9) | 13 ( 3.3) |
| Total | 97 (24.3) | 303 ( 75.7) | 400 (100) |

Pearson Chi-Square=0.674: *P<0.05*
One way-ANOVA=0.677: *P<0.05*

In terms of job, the lowest infection rate of *Toxoplasma* was in employee women (10%) and the highest in household women (25.6%). Statistical analysis showed no significant difference between these two variables. Since all women used the heath tap water, nobody had a history of receiving blood products, so chi-square test showed no significant correlation between type of accommodation (urban/rural), consumption of raw vegetables and type of housing (apartment /villa).

## Discussion

Toxoplasmosis is an important infection during pregnancy, because of abortion hydrocephaly, psychomotor disturbances, intracerebral calcification and chorioretinitis (12). Results of studies from

different parts of the world for toxoplasmosis have suggested different values (8-10) and Iran (11).

According to our study, out of 400 women prior to marriage, 97 cases (24.3%) were IgG positive and 303 cases (75.7%) were negative in comparison with other studies in different parts of Iran e.g. Kerman (34.5%) (13), Meshkin-shahr (18.3%) (14) and Ilam (44.8%) (15).

In the present study, 19 cases (4.8%) were IgG and IgM positive, it means that these cases have acute infection before the marriage. These cases have chronic disease and acquired immunity against *Toxoplasma* and 303 cases (75.7%) are IgM and IgG negative that they do not have innate or acquired immunity against *Toxoplasma*. If a mother is infected during pregnancy, it is the risk of transmission to fetus. Diagnostic tests should be done every 4-6 weeks for this case.

In this study, the correlation between education levels and anti-*Toxoplasma* IgG in serum was significant. The highest prevalence was seen in women with primary education (41.5%). This may be due to increasing of hygiene instructions and improved economy that educated people have minimal chance to face the source of infections. These findings are inconsistent with another study in Ramsar and Tonekabun (16).

Analysis of (one way-ANOVA) Turkey HSD showed that there were correlation between *Toxoplasma* infection with education levels of primary and high school.

In addition, women, who contacted with cats as pet animals, had higher antibodies titers in serum. This finding is inconsistent with the study of Meshkin shahr (14) but consistent with other studies (16, 17). Barbecue and grilled meat consumption can increase the rate of infection; it means that undercooked meat (under 70°C) could be a source of infection. However, meat is completely cooked and consumed in Arak, therefore raw liver, hamburgers may be a source for human infections in this city, as well cleaning meat without gloves, and handling of infected meats can be other ways of human infections. Results of the study in Gonabad (18) support our results. Type of residency (urban or rural) can affect prevalence of *Toxoplasma* infection but in our study, it did not affect. This study showed that the age groups of 18-23 and 28-33 yr had the most risk of infection but statistical analysis showed no significant differences among other age groups. The age range was influenced by the age of marriage.

## Conclusion

Raining rate in Arak is lower than north provinces (Gilan and Mazandaran) and cold season (prevention of oocysts development) is longer than some provinces. So, relative low prevalence of *Toxoplasma* infection was found in Arak City (24.3%) compared to other cities of Iran. Keeping cat as pet and consumption of undercooked liver and uncooked hamburger are the most important transmission routes for the infection. Since the majority of unmarried females that live in Arak City are *Toxoplasma* sero-negative (75.7%), using serological tests and health education prior to marriage or during pregnancy are recommended.

## Ethical consideration

Parents' agreements received before starting the research. In addition, special codes assigned instead the name of schoolchildren. Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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