

# Molecular Evaluation and Seroprevalence of Toxoplasmosis in Pregnant Women in Fars province, Southern Iran

Norouzi Larki Y<sup>1</sup>, Sarkari B<sup>2</sup>, Asgari Q<sup>1</sup>, Abdolahi Khabisi S<sup>1,3</sup>

<sup>1</sup>Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, <sup>2</sup>Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, <sup>3</sup>Department of Parasitology and Mycology, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

## Corresponding author:

Bahador Sarkari, Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran,  
Tel: 98-71-32305291; Fax: 98-71-32305291.  
E-mail: sarkarib@sums.ac.ir

## Abstract

**Background:** Importance of *Toxoplasma gondii* for humans refers mainly to primary infection in pregnant women and also infection in immunocompromised individuals. **Aim:** The current study aimed to determine the seroprevalence of toxoplasmosis in pregnant women in Fars province, southern Iran, and to find out the chronic and acute cases of toxoplasmosis in this population with molecular and serological methods. **Subjects and Methods:** Blood samples were taken from 2000 pregnant women, admitted to Shiraz university-affiliated hospitals in 2014 and serum and buffy coat were separated. Data such as age, number of pregnancy, pregnancy age and place of resident were recorded for each participant. Sera samples were evaluated for anti-*Toxoplasma* IgG and IgM, using a commercial ELISA kit. Moreover, the seropositive cases were evaluated by PCR to amplify a 529bp gene of *Toxoplasma gondii*. **Results:** Anti-*Toxoplasma* antibodies were detected in sera of 177 (8.9%) of cases. From these, 172 (8.6%) were seropositive only for IgG, 4 (0.2%) were seropositive only for IgM and 1 (0.05%) were positive for both IgG and IgM. PCR detected *Toxoplasma* DNA in buffy coat of 15 out of 177 (8.5%) of the seropositive subjects, two of them were IgM positive and the remaining 13 were among IgG seropositive cases. **Conclusion:** Findings of this study demonstrated a relatively low rate of seropositivity for toxoplasmosis in pregnant women in the area. This means that a high percentage of pregnant women are at risk of acquiring toxoplasmosis during their pregnancy and subsequently, transmission of the infection to their fetus.

**Keywords:** Seroprevalence, Molecular evaluation, Toxoplasmosis, Pregnant women, Southern Iran

## Introduction

*Toxoplasma gondii* is a cosmopolitan zoonotic protozoan which can infect many of warm blooded animals such as birds, mammals, and human.<sup>[1]</sup> Rate of human infection in different areas of the world is different due to variation in nutritional diets of the people, weather condition and reservoir hosts.

Infections with toxoplasmosis usually cause a mild disease in immunocompetent individuals and severe and life threatening infection in immunocompromised patient. The most serious manifestation of the disease can be seen in congenital toxoplasmosis, resulting from vertical transmission of the parasite during pregnancy.<sup>[2-4]</sup> Seroprevalence of toxoplasmosis in women at childbearing age varies in different geographical areas of Iran and ranged from 5 up to 70%.<sup>[5]</sup>

A prevalence rate of 55.5% has been reported form toxoplasmosis among pregnant women in north of Iran while this rate has been reported to be 29.3% in Khuzestan, south of Iran, with a different weather condition.<sup>[6-7]</sup> Fallah *et al.* reported a seroprevalence of 33.5% for *Toxoplasma* in pregnant women in 2004 in Hamedan, western Iran.<sup>[8]</sup> A seroprevalence rate of 34% was reported

for anti-*Toxoplasma* antibodies in pregnant women from Qazvin, in northwest of Iran.<sup>[9]</sup> Mostafavi *et al.* documented a seroprevalence of 47% in pregnant women in Isfahan, central Iran, and Davami *et al.* reported a seroprevalence of 15% for toxoplasmosis for women who referred to clinical laboratories for pre-marriage testing.<sup>[10,11]</sup>

It has been documented that *Toxoplasma* DNA can be detected in the buffy coat of individual during acute toxoplasmosis.<sup>[12]</sup> This might be a very useful approach for diagnosis of acute toxoplasmosis in pregnant women, where defining the acute infection in crucial for implementation of appropriate therapy and prevention measurements.

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The current study aimed to determine the seroprevalence of toxoplasmosis in pregnant women in Fars province, southern Iran, and to find out the chronic and acute cases of toxoplasmosis in this population with molecular and serological methods.

## Subjects and Methods

### Study population

The current study was conducted in 2014 in Fars province, southern Iran [Figure 1]. After getting approval from the ethics committee of Shiraz University of Medical Sciences, blood samples were taken from 2000 pregnant women admitted to Shiraz university-affiliated hospitals. Sample size was calculated based on the prevalence of toxoplasmosis in the region, using SPSS software (version 18, SPSS, Chicago). Demographic features and also number of pregnancy, pregnancy age and place of resident were recorded for each participant.

### Serological tests

Sera were obtained from the whole blood of each participant. Moreover, buffy coat was obtained from each blood sample for subsequent molecular study. Samples were transferred to the serology laboratory at department of parasitology and mycology in Shiraz University of Medical Sciences, Shiraz, Iran, and kept at  $-20^{\circ}\text{C}$  until use. All of sera samples were evaluated for anti-*Toxoplasma* IgG and IgM antibodies, using a commercial enzyme immunoassay kit (Pishtaz Teb Diagnostics, Tehran, Iran).

### Molecular analysis

The seropositive cases (either IgG or IgM) were evaluated by PCR to detect *Toxoplasma* DNA in the buffy coats. DNA was extracted from the buffy coat of each sample, using phenol chloroform method. PCR was performed to amplify a 529 bp gene of *T. gondii*, as described by Edvinsson *et al.* using each of two primers, TOXOF CAGGGAGGAAGACGAAAGTTG and TOXOR CAGACACAGTGCATCTGGATT.<sup>[13]</sup>



**Figure 1:** Map of Iran and location of Fars province (Dark red)

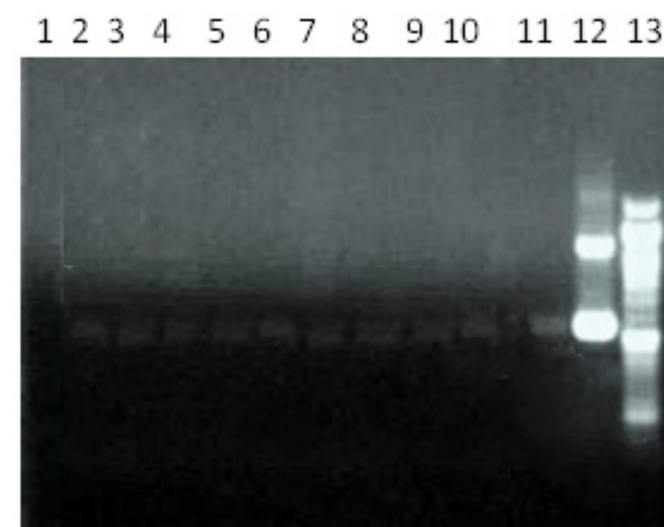
### Statistical analysis of the data

Collected data were analyzed by SPSS software (version 18, SPSS, Chicago). Fisher exact test and chi-square were used to compare the seroprevalence values related to the features of the subjects.

## Results

The pregnant women being studied were aged from 15 to 45 years old with mean age of 27.4 years. Most of the participants (35.4%) were aged 25-30 years old and the lowest (1.2%) were in the age group of 40 or higher. Most of pregnant women (57.3%) were in the second trimester of their pregnancy. Anti *T. gondii* antibodies were detected in the sera of 177 out of 2000 pregnant women, corresponding to an overall seroprevalence of 8.9% in this population. Of these, 172 (8.6%) were seropositive for only IgG, 4 (0.2%) were seropositive for only IgM and 1 (0.05%) was positive for both IgG and IgM.

Most of the seropositive cases (36.2%) were younger than 25 years old and the lowest (1.1%) were aged higher than 40 years old. Chi square test was used to find out the statistical association between seropositivity with number of pregnancy, trimesters of pregnancy, and age of the participants. There were a significant association between seropositivity and number and also trimesters of pregnancy of the participants ( $p < 0.01$ ). Most of IgM positive cases (0.3%) were seen in the age group of 15-30 years old whereas pregnant women aged 30-35 years had the lowest rate of IgM seropositivity. Correlation between IgM positivity and number of pregnancy or trimester were not statistically significant ( $p = 0.18$ ). All of seropositive cases were tested for the presence of *T. gondii* DNA in their buffy coat samples. Molecular study revealed a 529bp band of *T. gondii* in peripheral blood of 15 out of 177 (8.5%) seropositive pregnant women [Figure 2]. Two of these cases were IgM positive and the remaining 13 cases were among the IgG seropositive cases. Table 1 shows the demographic features of pregnant women and relative seropositivity to *T. gondii* in this study.



**Figure 2:** PCR product of 529 bp gene of *T. gondii* isolated from buffy coat of a few of pregnant women. Lane 1: negative control, Lane 2-11: samples isolated from the buffy coat of pregnant women, Lane 12: positive control (strain Rh of *T. gondii* (type I)), lane 13: Molecular marker

**Table 1: Demographic features of pregnant women and relative seropositivity to *T. gondii* in Fars province, southern Iran**

Characteristics	Frequency	Percent (%)	Positive for anti-Toxoplasma antibodies (either IgG or IgM)		P Value
			No	%	
<b>Age Group (Year)</b>					
<25	689	34.5	64	9.3	0.16
25-30	707	35.4	54	7.6	
31-35	448	22.4	38	8.5	
36-40	132	6.6	19	14.4	
>40	24	1.2	2	8.3	
<b>Pregnancy Age</b>					
First Trimester	23	1.2	6	26.1	0.001
Second Trimester	1146	57.3	139	12.1	
Third Trimester	831	41.6	32	3.9	
<b>Pregnancy Term</b>					
First	1030	51.5	109	10.6	0.007
Second	885	44.3	56	6.3	
Third	77	3.9	11	14.3	
Fourth	7	0.4	1	14.3	
Fifth	1	0.1	0	0	

## Discussion

Toxoplasmosis is a common parasitic infection of human and animals throughout the world, including Iran.<sup>[1,4,10,14]</sup> The overall seroprevalence rate of toxoplasmosis among the general population in Iran was reported to be 39.3%.<sup>[5]</sup>

In the current study seropositive rate of toxoplasmosis in pregnant women was found to be 8.8%, in southern Iran. This rate of seroprevalence in pregnant women is more or less similar to the rates reported in other studies conducted on child bearing and pregnant women in southern part of the country but lower than those reported from north part of the country.<sup>[6,7,9,15]</sup> Rate of *Toxoplasma* infection in north part of the country, where the temperature is ambient for survival of *Toxoplasma* oocyst excreted by the cat, is higher in comparison to the southern and western part of the country.<sup>[6,7,16]</sup>

Keeping cats and dogs as pets are not so common in Fars province in Iran. However, the number of stray cats and dogs are significant and stray cats, in this case, pose a major threat to human health through their role as reservoirs of *Toxoplasma*.

Consumption of contaminated vegetables might be the main source of *Toxoplasma* infection in pregnant women since in Iran women are usually clean and wash the vegetables at home and they may contaminate themselves while cleaning the vegetables. However, high seroprevalence of toxoplasmosis in meat producing animals in the region indicates that transmission may be occurring through consumption of undercooked meats of farm animals, as likely as contaminated vegetables.<sup>[17,18]</sup>

In the current study, rate of seropositivity was increased with pregnancy number where seropositivity was higher in multigravidae than in primigravidae. This is probably related to the age of the subjects rather than number of pregnancy, since the rate of seropositivity to *Toxoplasma* usually increase with the age.

The presence of IgM anti-*Toxoplasma* antibodies reflects the

risk of transplacental transmission. This is not always the case since IgM may persist as long as one year in sera of toxoplasmic patients. Therefore, other serological methods might be used to find out the estimate time of infection. Detection of *Toxoplasma* DNA in the buffy coat of the seropositive individuals may be related to acute infection. In a recent study in Fars province, south of Iran, 1500 cases of blood donors were evaluated for anti-*Toxoplasma* antibodies and 5.4% of the donors were seropositive for IgM and 1.6% for both IgG and IgM. *Toxoplasma* DNA has been detected in blood samples of two of IgM positive cases of blood donors but not in the buffy coat of the rest of seropositive cases.<sup>[14]</sup> In another study conducted on patients undergoing chemotherapy for malignancies in the Bushehr Province, Southwest Iran, *T. gondii* DNA was detected in the buffy coats of two out of 86 (2.3%) cases.<sup>[19]</sup> In the current study IgM anti-*Toxoplasma* antibody were detected in the sera of 5 (0.25%) of pregnant women while PCR detected *Toxoplasma* DNA in the buffy coat of 15 (0.75%) of the seropositive subjects, two of them were IgM positive and the remaining 13 were among IgG seropositive cases. Based on these findings, detection of *Toxoplasma* DNA in the buffy coat may not necessary indicate an acute infection. Moreover, presence of *Toxoplasma* DNA in the blood of IgM negative cases might be related to the false negativity of IgM result.

Taken together findings of this study showed that a low number of pregnant women in Fars province, southern Iran, are seropositive for toxoplasmosis. This indicates that high percentages of pregnant women are at risk of acquiring toxoplasmosis during their pregnancy and subsequent transmission of the infection to their fetus. Furthermore, in this study, molecular findings had no relationship with acute toxoplasmosis in studied cases. Further study, with large number of IgM positive cases, is needed to explore the value of PCR detection of *Toxoplasma* DNA in buffy coat for diagnosis of acute toxoplasmosis.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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