

## **Seroepidemiology of Toxoplasmosis in Women Referred To Ardabil Laboratory of Health Center for Medical Examinations Before Marriage, Iran, 2002**

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**Abstract:** Infection with *Toxoplasma gondii* can cause severe illness when the organism is transmitted to fetus or when it is reactivated in immune-suppressed individuals. As using the percentage of immune women who are going to get married is useful to determine the occurrence of abortion, still birth and foetal death to toxoplasmosis and congenital toxoplasmosis so the aim of this study was to determine the antibody prevalence of toxoplasmosis in women referred to laboratory of health center for medical examinations before marriage. This cross-sectional study was performed on 504 sera collected from women in Ardabil city, Iran, in 2002. The samples were studied by indirect immunofluorescent assay (IFA) for determination of IgG and IgM antibodies to toxoplasma. The seroprevalence of IgG antibody at a titer of  $\geq 1:20$  was 34.7%. There was the most of antibody titer frequency in 1:20 titer (11.7%) and the least of them in 1:3200 (0.4%) and 1:6400 (0.4%) titres. Eighty eight percent of seropositive women consumed vegetables, 67.5 and 61.6% of them contacted with raw meat and infected cats, respectively. Only 20 individuals (4%) showed IgM antibody against *Toxoplasma gondii*. No statistically significant differences in the levels of antibodies were observed in relation to age, history of contact with cat or domestic animals. Since 65.3% of women before marriage in Ardabil city were seronegative and the risk of abortion, still birth, foetal death due to toxoplasmosis and also congenital toxoplasmosis is high so health education to omit its risk factors, especially in women who are going to marry and also during pregnancy is necessary.

**Key words:** Seroepidemiology, toxoplasmosis, ardabil laboratory, HCME

### **INTRODUCTION**

*Toxoplasma gondii* is spread worldwide and infects human as well as several other mammals. Prevalence of antibodies to *T. gondii* in the world appears to be 33%<sup>[1]</sup>. In Iran, its prevalence among the inhabitants of the Caspian Sea area is relatively high (55.7%)<sup>[2]</sup>. There is much evidence that the habit of consumption of undercooked or raw meat is related to high toxoplasmosis morbidity. Toxoplasmosis may result from contact with food, water or sand contaminated with oocysts spread by infected cats. There is also the possibility of transplacental transmission of the parasite to the fetus during pregnancy. Congenital toxoplasmosis develops in about half of pregnant women who were primarily infected during pregnancy. The parasites may cause intracranial calcifications, hydrocephalus and chorioretinitis<sup>[3]</sup>. In people with a well-functioning immunologic system the infection usually has no symptoms except for

development of specific antibodies. In people with immunologic disorder, primary invasion may develop or recurrence of chronic invasion may develop or recurrence of chronic invasion with clinical signs. Most common is the lymphonodular form of toxoplasmosis involving mainly lymph glands in the neck and nape area. Changes may concern also liver, lungs and cardiac muscle, central nervous system and eyes<sup>[3]</sup>. Encephalitis<sup>[4]</sup> and pneumonia cases<sup>[5]</sup> have been increasing in patients with acquired immunodeficiency syndrome (AIDS). The aim of the present study was an evaluation of the frequency of anti-*Toxoplasma gondii* antibodies in women referred to laboratory of health center for medical examinations before marriage.

### **MATERIALS AND METHODS**

This cross-sectional study was performed on 504 sera collected from women referred to Ardabil laboratory of

Table 1: Distribution of IgG antibody to *Toxoplasma gondii* in women 15 - 45 years old referring to laboratory of health center, Ardabil, Iran (2002)

Seropositive with titre	Frequency	
	No.	%
1:20	59	11.8
1:10	48	9.5
1:200	28	5.5
1:400	23	4.6
1:800	8	1.6
1:1600	5	0.9
1:3200	2	0.4
1:6400	2	0.4
Total	175	34.7

Table 2: Distribution of seropositive cases based on epidemiological information in women 15-45 years old referring to laboratory of health center, Ardabil, Iran (2002)

Epidemiological information	No. cases	Seropositive cases	
	No. Total	No.	%
Contact with raw meat	336/498	116	34.5
History of enlarging lymph nodes	66/500	20	30.3
Vision position	104/502	45	43.3
Keeping cat in house	90/502	26	28.9
Keeping cattle in house	202/503	62	30.7

health center for examinations before marriage, in Ardabil City, Iran, 2002. The samples were studied by Indirect Immunofluorescent Assay (IFA). This test detects specific antibodies of IgG and IgM and was carried out as follow:

The fresh antigen was prepared in our laboratory. Tachyzoites from 3 day peritoneal exudates of mice inoculated with RH strain of *Toxoplasma gondii* were centrifugated at 3,000 rpm and formalinized (1%) for 90 min. After centrifugation, sediment was suspended in PBS (pH 7.6). Then antigen was diluted with PBS (until 10-30 tachyzoites were visible in the field of view of the microscope at 400× magnification) and dripped on glass slides, air-dried and stored at -20°C until needed. Before use, antigen-coated slides were rinsed with water and repeatedly air-dried. Tested sera were placed on antigen spots (15µL/spot) in two dilutions (1:20 and 1:100) and incubated at 37°C for 30 min. The slides were then rinsed in water, washed in PBS (pH 7.6) three times for 5 min each, and dried. A drop of fluorescein isothiocyanate labeled goat antihuman immunoglobulin diluted in PBS (in proportion established by titration) was added to each spot. An addition of equal volume of 0.01% Evans blue to immunoglobulin helped in later visualization of *Toxoplasma* fluorescence. Slides were incubated at 37°C for 30 min, washed, dried and mounted with buffered glycerol. Slides were examined using a fluorescent microscope. Positive titres were greater or equal to 20. Serum positive in both dilutions was examined in higher dilutions to establish endpoint titre.

Table 3: Distribution of IgG antibody to *Toxoplasma gondii* by age, in women 15 - 45 years old referring to laboratory of health center, Ardabil, Iran (2002)

Age groups	No. tested	Seropositive		Seronegative	
		No	%	No	%
<20	269	94	34.9	175	65.1
≥21	231	77	33.3	154	66.7
Total	500	171	34.2	329	65.8
X <sup>2</sup> =0.14		.F=1		P>0.05	

## RESULTS

One hundred and seventy five (34.7%) of 504 samples examined showed positive titers higher than 1:20 by IFA. There was the most of antibody titer frequency in 1:20 titer (11.7%) and the least of them in 1:3200 (0.4%) and 1:6400 (0.4%) titres (Table 1). 88% of seropositive women consumed vegetables, 67.5% and 61.6% of them contacted with raw meat and infected cats, respectively (Table 2). Statistical analysis has not shown a significant difference between distribution of toxoplasmosis frequency and history of enlarging lymphatic glands, way of washing vegetables, contact with raw meat, washing hands before eating food or contact with domestic cats. No significant difference in the seropositive rate was observed between persons under 20 years old and upper than 20 (Table 3). Only 20 persons (4%) showed IgM antibody against *Toxoplasma gondii* (Table 4).

## DISCUSSION

Toxoplasmosis has a wide geographical distribution. It is a serious health problem in various countries<sup>[6]</sup>. The present study showed that 125 of 504 people (34.7%) were seropositive against *T. gondii*. The positive rates of anti- *Toxoplasma* antibody in different parts of Iran vary: Roudsar 86.3%, Meshkinshahr 18.3%, Sari 71%, Rafsanjan 48%, and Sardasht 6.3%<sup>[7]</sup>. The prevalence of *T. gondii* in Ardabil City was similar to some of cold countries such as Czech<sup>[8]</sup>. The frequency of infection varies from one country to another and also in different parts of a country<sup>[9]</sup>. This variation is presumably due to the presence or absence of cats, climatic, hygienic, and socioeconomic conditions and consumption of raw or improperly cooked meat<sup>[10,11]</sup>. Of the 2 main modes of *Toxoplasma* transmission – by infective cat faeces or tissue cysts from undercooked meat – the second might play a predominant role in the spread of human infection in this population.

This study has been performed on women 15-45 years old, and no statistically difference was observed in women <20 and ≥20 years.

Table 4: Distribution of IgM antibody to *Toxoplasma gondii* in women 15-45 years old referring to laboratory of health center, Ardabil, Iran(2002)

Ab titre	Frequency	
	No.	%
Negative	155	88.6
1:10	5	2.8
1:20	11	6.3
1:100	4	2.3
Total	175	100

Of course contrary to this study, seroprevalence of toxoplasmosis is known to increase with age. The reason for the rise in quantitative titers with age is not clear. A hypothesis would be that the increase is a reflection of increasing exposure years as the humans get older. Multiple minor infections might at first produce low antibody levels and later higher levels<sup>[12]</sup>.

Although no statistically significant differences in the levels of antibody were observed in relation to history of contact with domestic cats and keeping domestic animals in the house, it seems to some risk factors of toxoplasmosis in this group of women were poor hand hygiene, consumption of undercooked beef, domesticating a pet cat, frequent consumption of raw vegetables outside the home and the consumption of undercooked lambs.

The findings of this study and the known food habits of the study population suggest ingestion of *Toxoplasma* cysts in meat to be the main mode of transmission of the disease. In Europe and the USA, toxoplasmosis is acquired mainly by the ingestion of undercooked meat, as has been noted in surveys from France, North America and Norway<sup>[13]</sup>.

As 65.3% of these women were seronegative, and they will be at high-risk for first infection during pregnancy, so health education to omit its risk factors, especially during the pregnancy is necessary; because when pregnant women acquired primary infection with *T. gondii* especially during their second or third trimester, they can transmit the infection transplacentally to their fetus<sup>[14]</sup>. The infected babies may develop congenital toxoplasmosis and suffer from symptoms such as chorioretinitis, convulsions, jaundice, hydrocephalus, fever, pneumonitis, hepatosplenomegaly, lymphadenopathy, microcephalus, cataract, hypothermia, and rash<sup>[15]</sup>. Up to 75% of the infected babies have no clinical manifestations<sup>[16]</sup>, and most toxoplasmic children develop clinical manifestations several years after birth<sup>[17]</sup>.

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