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Original Article

Serological Assessment of *Toxoplasma* Infection by ELISA method in Urban People and Livestock Farmers of Tabriz, Iran

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Abstract

Introduction: Toxoplasmosis is a common infection between humans and animals in the world. In its congenital form, the causative agent of the disease is transmitted through the mother's placenta to the fetus. Acquired infection is caused by eating oocysts that the cat excretes with feces or through contaminated meat. In people who have problems with the immune system, in malignant patients, in people who do organ transplants, and in patients with AIDS, the infection may be acute and fatal. This study aimed to investigate the prevalence of IgM and IgG anti-*Toxoplasma gondii* antibodies in the blood serum of urban residents and livestock farmers of Tabriz, Iran, in 2021.

Methods: The blood of 50 urban residents and 50 livestock farmers was taken for sampling. The patient's arm vein was used for blood sampling. Then, the blood was kept at 37 degrees Celsius for 15 minutes and centrifuged (2000 rounds for 10 minutes). After centrifugation, the sera were carefully poured into a microtube and frozen and kept for an enzyme-linked immunosorbent assay (ELISA) test.

Results: The study of the frequency distribution of toxoplasmosis by ELISA method in urban residents and livestock farmers in Tabriz and suburbs showed that out of 100 studied people, 36 cases (36%) of the total had anti-toxoplasma IgG titer higher than normal (positive). In the group of positive cases of toxoplasmosis in livestock farmers, 24 (48%) out of 50 cases had a higher-than-normal titer, which is considered positive. Further, out of the 50 urban people surveyed, 12 cases (24%) had titers higher than normal, which is considered positive. In the study of the frequency distribution of anti-toxoplasma IgM antibody by ELISA method in urban people and livestock farmers, 36 positive cases and 64 negative cases were observed out of 100 people, and15 out of 50 urban people (i.e., 30% of urban people) had IgM titer higher than normal. Moreover, an examination of 21 people out of 50 livestock farmers (42%) indicated higher than normal IgM titers.

Conclusion: This study indicated that IgG and IgM antibody titers are higher in livestock farmers than in urban residents, and this suggests a relationship between occupation and toxoplasma infection. Therefore, the need is raised for extensive research at the national level to reduce the disease in livestock farmers and urban residents.

Keywords: *Toxoplasma*, Urban people, Livestock farmers, ELISA, Tabriz

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Introduction

Since the existence of humans, there has been a close relationship between man and animals, which is either for providing food, protecting and guarding life, or meeting their emotional needs. Therefore, in addition to meeting the needs of man, this close relationship creates a problem for humans and sometimes for animals. Among these problems, we can refer to the important diseases common between humans and animals, some of which cause fatal infections in both the animal owner and the animal itself (1). Toxoplasmosis is a common disease whose final host is the cat, and its intermediate host is humans and other mammals. This disease also causes abortion in animals and induces a lot of damage (2).

This disease does not cause many problems in cats, but it creates many problems in intermediate hosts such as humans, especially in pregnant women and people with weakened immune systems. *Toxoplasma gondii* is an obligate intracellular parasite with worldwide distribution and belongs to the order Coccidia (3). Serological information shows that 20% to 75% of different populations are chronically but asymptomatically affected by the tissue cyst form of this infection (4). As long as the body's immune system is active, the parasites appear as

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cysts (chronic form), but if the body's immune system is reduced or impaired, it will become active, and its clinical symptoms can be revealed. Considering the increasing incidence of acquired immunodeficiency syndrome (AIDS) and cancer in the world, in which the immune system is weakened and considering that cancer patients and those receiving organ transplants use drugs that weaken the immune system, and toxoplasmosis and other opportunistic secondary infections appear. Accordingly, this study was conducted to identify the parasite in these people as much as possible (5,6). In this study, an attempt was made to investigate the level of *Toxoplasma* infection in livestock farmers and urban residents in Tabriz and its suburbs to obtain information on the serum levels of this parasite and the frequency of people infected with this disease. It is hoped that other efforts will be made in other cities to acquire complete information about the incidence of this disease among different people. The present study was carried out with regard to the mentioned problems in order to increase awareness of the epidemiology of toxoplasmosis in Tabriz in 2021.

Materials and Methods

The participants included 50 urban residents and 50 livestock farmers who had their blood taken for sampling. The patient's arm vein was used for blood sampling. Then, the blood was kept at 37 degrees Celsius for 15 minutes and centrifuged (2000 rounds for 10 minutes). After centrifugation, the sera were carefully poured into a microtube and frozen and kept for an enzyme-linked immunosorbent assay (ELISA) test (Figures 1 and 2).

To conduct the ELISA tests, the IgG and IgM anti-*T. gondii* IgG and IgM ELISA test kits were used according to the protocol of the Pishtaz Medical Company. To evaluate the results of the ELISA test, we read the optical absorption of the standards and samples with the help of an ELISA reader at a wavelength of 450 nm. The current test kits have the ability to measure IgM and IgG antibodies against *Toxoplasma* with high sensitivity and specificity. For quantitative calculations, a standard curve diagram was drawn using the average optical absorption of the standards and their known concentration. In a normal population, the cut-off value is the standard equivalent of 10 IU/mL. Values below this curve are considered negative, and values above this value are considered positive.

Statistical Methods

SPSS statistical software was used to analyze the data, which is described by central indicators, and χ^2 statistical tests were also employed.

Results

The study of the distribution of the frequency of toxoplasmosis by ELISA method in urban people and livestock farmers in Tabriz, Iran, and suburbs indicated that out of 100 people, 36 people (36%) of the total were positive and had anti-toxoplasma IgG titer higher than normal (Table 1). Moreover, in the group of positive cases of livestock farmers, 24 out of 50 people (48%) had a higher-than-normal titer, which is considered a positive titer. Likewise, 12 cases (24%) out of the 50 surveyed urban people had titers higher than normal, which is considered positive.

In the investigation of the frequency distribution of anti-*T. gondii* IgM antibody by ELISA method in urban livestock farmers, out of all 100 examined people, 36 positive cases and 64 negative cases were observed, as illustrated in Table 2. Further, 15 cases out of 50 urban people (i.e., 30% of urban people) had IgM titer higher than normal. Additionally, as observed in Table 3, 21 cases out of 50 livestock farmers (42%) had IgM titers higher than normal, 12 out of 50 (24%) urban people had a higher than normal IgG titer, and 24 out of 50 (48%) livestock farmers, had IgG titer higher than normal.

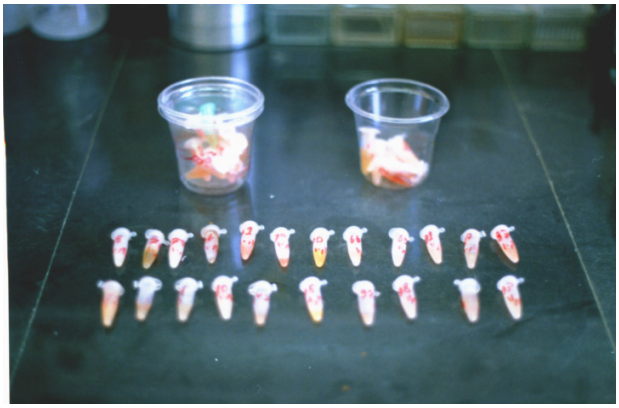

Figure 1. Sera Collected From Patients Inside a Microtube

Figure 2. The Stages of Doing ELISA Tests With the ELISA Device. *Note*. ELISA: Enzyme-linked immunosorbent assay

Table 1. Distribution of the Frequency of Toxoplasmosis in Urban People and Livestock Farmers According to IgG

	Positive		Negative		Total	
	Number	%	Number	%	Number	%
Urban people and Livestock farmers	36	36	64	64	100	100
Total	36	36	64	64	100	100

Table 2. The Frequency of IgM Anti-*Toxoplasma* Antibodies in Urban People and Livestock Farmers

	Number	Positive Percentage
Anti-*Toxoplasma* IgM antibody in urban people	100	30
Anti-*Toxoplasma* IgM antibody in livestock farmers	100	42

Table 3. The Frequency of Anti-*toxoplasma* IgG Antibody in Urban People and Livestock Farmers

	Number	Positive Percentage
Percentage frequency of anti-*Toxoplasma* IgG antibody in urban people	100	24
Percentage frequency of anti-*Toxoplasma* IgG antibody in livestock farmers	100	48

Discussion

The rate of positive cases of anti-toxoplasma IgG antibody in urban people and livestock farmers of Tabriz was determined to be 24% and 48%, respectively, using the ELISA method. The comparison of this ratio with the studies conducted in other regions of Iran indicates a relatively smaller difference (7,8).

In the present study, which was conducted on 100 people, 36 cases (36%) had appreciable anti- *T. gondii* IgG titer. In a study in Thailand, the amount of specific anti- *T. gondii* IgG and IgM antibodies in healthy people was reported to be 2.3% and 0%, respectively. In another study in which 108 people were used as the control for cancer patients, 19.4% and 0.9% had significant positive IgG and IgM titers, respectively (9,10). Moreover, Martino and colleagues reported that out of 41 cases of patients who underwent bone marrow transplantation in the transplant center, 94% were positive for *T. gondii* before transplantation, and 73% had the disease before undergoing bone marrow transplantation, which was moderate to severe (11).

In this study, which was conducted on urban people and livestock farmers, 36% had a significant positive titer for *Toxoplasma*, indicating a difference compared with the studies of Martino et al. In other words, infection is more common in hot climates and low-altitude areas than in cold climates and mountainous areas, and it is more common in wet areas than in dry areas. Probably, this situation depends on the suitable conditions for sporulation and survival of oocysts in the environment because sporulation stops at temperatures above 37°C and below 4°C, and oocysts remain active in moist soil for more than a year, but they lose their infectivity power in brightness and heat more than 66 degrees centigrade. Cultural habits and the health of people may also play a role. In general, infection is less common in cold and mountainous regions and very hot regions (12). This can justify the difference of positive percentages in the study by Martino and colleagues in Europe and the current study.

Some studies did not find a significant relationship between gender and the prevalence of infection (13). In this study, the prevalence of toxoplasmosis in urban people and livestock farmers was 24% and 48%, respectively, which is statistically significant and shows a relationship between occupation and toxoplasma infection. In America, the prevalence of antibodies increased by one percent for every year of increase in age (14,15).

In a study that was conducted on bone marrow transplant recipients and donors from 2001 to 2004, 220 donors and transplant recipients were tested for *Toxoplasma* serology, and the results indicated that eight people were donors and recipients of toxoplasma-positive serum, and 59 transplant recipients were seropositive (high risk) for toxoplasma. In addition, 12 people were positive donors for transplant, and 141 people were negative donors and negative recipients for *Toxoplasma*. In total, 67 people (30%) were diagnosed as high risk for *Toxoplasma* infection (16,17).

The results of this study reveal the prevalence of *Toxoplasma* (24% in urban people and 48% in livestock farmers), and the study by others shows a 30% prevalence. In a study conducted by Arbabi et al in 1993, the prevalence of infection in urban areas and in the entire urban community was estimated to be 47.6% and 44.8-50.4%, respectively (18).

In another study, Saeedi et al investigated seroepidemiology of anti-*Toxoplasma* antibodies in women referred for marriage counseling and found a significant relationship between IgM positivity and contact with cats. In addition, this study considered the relationship between the consumption of unboiled milk and the positivity of anti-*Toxoplasma* antibody, and finally, a significant relationship was obtained between the consumption of unboiled milk and the positivity of IgG (19). This reflects the concordance of this study with our study, so the possibility of consuming unboiled milk is high in livestock farmers; hence, the antibody titer against *Toxoplasma* is higher in livestock farmers compared to urban people, and this issue was confirmed in the present study.

Suggestions

Considering the importance of long life in humans and the need for more help to maintain the health of humans, it is suggested to carry out a general investigation on the presence of *Toxoplasma* among these people, which

turns from chronic to acute form. *Toxoplasma* detection in people is very important and should be implemented in all countries. It is better for the Ministry of Health to allocate a budget for research on *Toxoplasma* and use more accurate methods than ELISA such as polymerase chain reaction (PCR) and parasite isolation from blood or body fluids (e.g., parasite isolation and tissue examination), which are considered powerful diagnostic methods. The specificity and value of the positive PCR result is almost 100%, while the specificity of the ELISA test is 96.4%. The PCR method is not routinely performed today due to its time-consuming and expensive nature. In less developed places, previous methods such as indirect immunofluorescence can be used for the serological investigation of *Toxoplasma*. In the present study, due to the limited time and space, the information review is valid for the same range and specific time, but to extend the work, future studies should be conducted at different times and places such as hospitals and universities and among pregnant women and high-risk people.

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Conflict of Interests

The authors of the article declare that they have no conflict of interests.

Ethics Issues

All medical ethical rules have been fully observed in this study.

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