Research Article

*Toxoplasma gondii* in Blood Donors: A Study in Boyer-Ahmad County, Southwest Iran

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**1. Introduction**

*Toxoplasma gondii* is often a water and foodborne pathogen with a wide and considerable distribution which can be transmitted through blood transfusion and causes acute severe complication such as encephalitis in immunocompromised blood recipients [1, 2]. Toxoplasmosis may result in abortion or neurological abnormalities in human fetus following intrauterine transmission [1, 3]. Toxoplasmosis is an asymptomatic mild infection in most of the immunocompetent individuals, yet a number of studies have shown a significant association between latent toxoplasmosis and psychiatric disorder among immunocompetent individuals [4–6].

The main routes of *T. gondii* infection in human are ingesting of sporulated oocysts in contaminated water, vegetables, and fruits along with eating undercooked contaminated meat and also vertical transmission at the time of pregnancy. Moreover, the healthy human can be infected through blood transfusion or organ transplantation. Toxoplasmosis is a common infection in human and animals in all areas of Iran [2, 7–11]. Blood donors, especially those who are in the acute phase of the infection, can impose risk of *T. gondii* infection for the susceptible recipients [12, 13]. The mean prevalence of toxoplasmosis among Iranian blood donors varied between 12.3% and 52.8% [14]. Recent studies reported anti-*T. gondii* IgG antibodies in sera of 19.3% of blood donors, 8.5% of...
female university students, and 8.9% of pregnant women in southern Iran [2, 15, 16].

The current study was performed to assess the seroprevalence rate of *T. gondii* and possible associated risk factors among blood donors in Boyer-Ahmad in southwest Iran.

### 2. Materials and Methods

#### 2.1. Study Area

This cross-sectional study was conducted on healthy blood donors of Boyer-Ahmad county, located in southwest Iran, from April to August 2015. Boyer-Ahmad is located in a mountainous and cold region of Iran. Snow and rainfall are plentiful in this region, especially in fall and winter seasons. The mean altitude in the area where the Boyer-Ahmad people live is 1800–2200 m above the sea level. The area has geographical coordinates between latitudes 30–9° and 31–27° N and between longitudes 49–55° and 51–42° E. Animal husbandry and gardening are common in this district. Food habits of more people are composed of meat, milk, and local vegetables. Parasitic diseases are not uncommon in this area and the area is considered as a focus of human fascioliasis as well as visceral leishmaniasis [17–21]. Also, high levels of blood-borne diseases including hepatitis C and B have been reported from the area [22, 23].

#### 2.2. Sampling

Subjects of this study were recruited from healthy blood donors who knowingly agreed for participation in the study. All participating individuals were given a questionnaire before collection of blood sample, which provided information about their age, sex, residence area, level of education, occupation, and risk factors including the consumption of raw/undercooked meat, contact with cat, contact with soil in gardening or agricultural activities, and history of blood transfusion. Awareness about toxoplasmosis and possible associated risk factors for toxoplasmosis in the population, but the seroprevalence rate of toxoplasmosis in the current study indicates a considerable rate of toxoplasmosis in the population, but the seroprevalence rate of toxoplasmosis in our study is lower than the rates reported from other studies in the region [24].

#### 2.3. Serologic Testing

All of the collected sera were tested for detection of anti-*T. gondii* IgG and IgM antibodies, using a commercial ELISA kit (ACON Biotech, Hangzhou, China), based on the manufacturer's instructions. Index value was obtained for both IgG and IgM. An index value ≤ 0.9 IU/mL was regarded as negative result, while the equivocal range was defined between 0.9 and 1.1 IU/mL and index value greater than 1.1 IU/mL was considered as positive result for both IgG and IgM.

#### 2.4. DNA Extraction and PCR Amplification

DNA was isolated from the buffy coat of each sample, using the phenol-chloroform extraction method as previously described [2]. The conventional PCR for detection of *T. gondii* DNA was performed targeting a 529 bp gene, with primers TOXOF (5'-CAGGGAGGAAGACGAAAGTTG-3') and TOXOR (5'-CAGACACAGTGCACTTGGATT-3') [24]. The total reaction volume was 25 μL, containing 1 unit of Taq polymerase, 12.5 μL of 2x Master Mix Red, 1.5 mM of MgCl₂, 1 μL of each 20-picomole primer, 50 ng of extracted DNA, and the remaining nuclease-free water. The PCR program was set for 5 minutes at 94°C before cycling, followed by 30 cycles of denaturation at 94°C for 35 seconds, annealing at 56°C for 1 minute, extension at 72°C for 1 minute, a final extension at 72°C for 10 minutes, and final hold at 4°C for 10 minutes. Amplification was performed with negative along with positive control of *T. gondii* (a kind gift of Dr. Q. Asgari). The 529 bp PCR products were separated by electrophoresis in 1.5% agarose gel and stained with ethidium bromide.

#### 2.5. Statistical Analysis

SPSS 18 software was used for all statistical analyses. The frequency of dependent variables was described, using descriptive statistics, and chi-squared and regression logistic tests (*p* < 0.05) were used to find out any possible association between qualitative variables and seropositivity to toxoplasmosis.

### 3. Results

#### 3.1. Demographic Features of the Participants

Overall, 285 healthy blood donors were recruited in this study. The mean age of the subjects was 37 (±9.53) years. Most of the blood donors (41.8%) were in the age group of 28–37 years. The majority of the subjects were male (96.8%). Most of the subjects (36.5%) were employees.

#### 3.2. Seroprevalence of Anti-*T. gondii* Antibodies

Anti-*T. gondii* antibodies were detected in sera of 48 out of 285 blood donors. Of these, 46 cases (16.30%) were seropositive only for IgG and 2 cases were seropositive for both IgG and IgM. Seropositivity in males and females was 16.3% and 11.1%, respectively.

#### 3.3. Risk Factors for *T. gondii* Seropositivity

In the univariate analysis, three variables including contact with soil (*p* = 0.01), consumption of undercooked meat (as barbecue or kebab), (*p* = 0.007), and job (*p* = 0.040), were documented as associated risk factors for *T. gondii* seropositivity. Other risk factors and also demographic features of the blood donors were not statistically associated with the acquisition of *T. gondii* infection (*p* ≥ 0.05) (Table 1).

#### 3.4. Detection of *T. gondii* DNA in Seropositive Subjects

None of the 48 seropositive subjects were positive for *T. gondii* by molecular (PCR) method.

### 4. Discussion

This is a cross-sectional study regarding the seroprevalence and molecular evaluation of *T. gondii* infection among healthy blood donors of Boyer-Ahmad County in southwest Iran. The overall seroprevalence of 16.8% for toxoplasmosis in the current study indicates a considerable rate of toxoplasmosis in the population, but the seroprevalence rate of toxoplasmosis in our study is lower than the rates reported
Table 1: Risk factors analysis of seropositivity to toxoplasmosis among blood donors in southwest Iran.

| Variables                      | Frequency (number) | Seropositivity (%) | Odds ratio (95% confidence interval) | p value |
|--------------------------------|--------------------|--------------------|--------------------------------------|---------|
| Blood transfusion              |                    |                    |                                      |         |
| Yes                            | 8                  | 37.5               | 3.09 (0.714–13.407)                  | 0.135   |
| No                             | 277                | 16.2               | 1                                    |         |
| Place of residence             |                    |                    |                                      | 0.602   |
| City                           | 203                | 17.5               | 1.257 (0.618–2.559)                  |         |
| Village                        | 82                 | 14.6               | 1                                    |         |
| Eating of semicooked meat      |                    |                    |                                      | 0.007   |
| Yes                            | 157                | 22.30              | 2.538 (1.278–5.038)                  |         |
| No                             | 128                | 10.15              | 1                                    |         |
| Contact with cat               |                    |                    |                                      | 0.841   |
| Yes                            | 51                 | 15.6               | 0.902 (0.394–2.065)                  |         |
| No                             | 234                | 20.51              |                                      |         |
| Taking immunosuppressive drugs |                    |                    |                                      | 0.199   |
| Yes                            | 5                  | 40                 | 3.391 (.551–20.866)                  |         |
| No                             | 280                | 16.4               |                                      |         |
| Contact with soil              |                    |                    |                                      | 0.001   |
| Yes                            | 61                 | 47.5               | 9.778 (4.914–19.457)                 |         |
| No                             | 224                | 8.5                |                                      |         |
| Job                            |                    |                    |                                      | 0.040   |
| Employees                      | 112                | 25                 | 1                                    |         |
| Other business                 | 81                 | 16                 | 1.744                                |         |
| Student                        | 24                 | 4                  | 7.667                                |         |
| Farmer and rancher             | 55                 | 9.09               | 3.333                                |         |
| Unemployed                     | 13                 | 7.70               | 4.000                                |         |
| Educational level              |                    |                    |                                      | 0.592   |
| Secondary level and below      | 56                 | 17.86              | 1.049 (0.469–2.348)                  |         |
| High-school level              | 89                 | 13.5               | 1.463 (0.696–3.075)                  |         |
| University level               | 140                | 18.6               | 1                                    |         |
| Blood group                    |                    |                    |                                      | 0.285   |
| A                              | 88                 | 13.6               | 1.715 (0.812–3.621)                  |         |
| B                              | 57                 | 15.8               | 1.444 (0.628–3.324)                  |         |
| AB                             | 18                 | 5.5                | 4.604 (0.585–36.226)                 |         |
| O                              | 122                | 21.3               | 1                                    |         |
| Washing vegetables with antiseptic materials | | | | 0.587 |
| Yes                            | 73                 | 19.7               | 1                                    |         |
| No                             | 212                | 16.03              | 1.242 (0.624–2.473)                  |         |
| Awareness about toxoplasmosis  |                    |                    |                                      | 0.702   |
| Yes                            | 10                 | 10                 | 0.539 (0.067–4.357)                  |         |
| No                             | 275                | 17.1               | 1                                    |         |

in blood donors from most of the areas in Iran [2, 7, 25] and other regions as reported from Turkey (22.59%) [26], Czech Republic (34.23%) [27], Brazil (60%) [28], Saudi Arabia (40%) [29], Iraq (32.75%) [30], Egypt (59.6%) [31], and India (53.7%) [32]. Variations in the rate of seropositivity of *T. gondii* in different regions of the world or in different areas of a given country can be attributed to the differences in climate, topographical conditions, and food behavior.

Lower seroprevalence of toxoplasmosis in high altitude and cold climates has been reported, as *T. gondii* oocysts cannot survive for a long time in such environmental conditions. In the present study, consumption of undercooked meat was identified as a risk factor related to *T. gondii* seropositivity in blood donors. These findings indicate that the ingestion of undercooked meat, mainly sheep and goat, containing tissue cysts might be one of the main sources of *T. gondii* infection in
this area [33, 34]. This notion has already been documented in other areas of Iran [8, 10]. Exposure to the soil, followed by oocysts in soil, is an important risk factor for acquisition of T. gondii. Risk factors of toxoplasmosis vary in different geographic regions. Contact with cats in Mexico and eating raw shellfish as well as exposure to domestic cats in Taiwan [35] have been counted as the main risk factors for T. gondii infection among the blood donors. In rural communities of northern Iran, consumption of undercooked sheep and goat meat and unwashed raw vegetables or fruits have been considered as the main risk factors for T. gondii infection [34], while contact with cats and consuming raw vegetables and raw milk/egg were identified as independent risk factors for T. gondii seropositivity among the healthy blood donors in the southeast of the country [36].

In the current study, there was a significant correlation between job and seropositivity with T. gondii. This fact can be justified as most of the people in the studied area live on animal husbandry and agricultural activities, which increases the chance of T. gondii infection. None of the seropositive cases were positive for T. gondii DNA. This indicates that all of the seropositive cases have been in the chronic phase of toxoplasmosis and have no risk of T. gondii infection for the recipients.

This study showed that more than 16% of the healthy blood donors in Boyer-Ahmad County in southwest Iran have anti-T. gondii antibodies in their sera and the consumption of undercooked meats, job, and contact with soil were three independent risk factors associated with T. gondii infection.

Ethical Approval
The study was approved by the Ethical Committee of Yasuj University of Medical Sciences (YUMS).

Consent
Verbal informed consent for the study was obtained from all participants prior to enrolment.

Disclosure
The study was the subject of Mr. Saadat Kazemi’s M.D. thesis.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Bahador Sarkari, Abdolali Moshfe, and Ahmad Mardani conceived and designed the study. Saadat Kazemi and Nasir Arefkhah collected the samples. Nasir Arefkhah and Saadat Kazemi performed the experiments. Bahador Sarkari, Ahmad Mardani, and Nasir Arefkhah analyzed the data. Nasir Arefkhah wrote the first draft of the paper. Bahador Sarkari edited the paper. All authors read and approved the final manuscript.

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References
[1] J. Flegr, J. Prandota, M. Soviˇckov´a, and Z. H. Israili, ”Toxoplasmosis - A global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries,” PLoS ONE, vol. 9, no. 3, Article ID e90203, 2014.
[2] B. Sarkari, R. Shafei, M. Zare, S. Sohrabpour, and L. Kasraian, ”Seroprevalence and molecular diagnosis of Toxoplasma gondii infection among blood donors in southern Iran,” The Journal of Infection in Developing Countries, vol. 8, no. 4, pp. 543–547, 2014.
[3] B. Sarkari and S. A. Habis, ”Severe congenital toxoplasmosis: a case report and strain characterization,” Case Reports in Infectious Diseases, vol. 2015, Article ID 851085, 3 pages, 2015.
[4] L. M. Weiss and J. P. Dubey, ”Toxoplasmosis: a history of clinical observations,” International Journal for Parasitology, vol. 39, no. 8, pp. 895–901, 2009.
[5] J. P. Dubey and J. L. Jones, ”Toxoplasma gondii infection in humans and animals in the United States,” International Journal for Parasitology, vol. 38, no. 11, pp. 1257–1278, 2008.
[6] A. Rostami, H. Keshavarz, S. Shojaee, M. Mohebali, and A. R. Meemar, ”Frequency of Toxoplasma gondii in HIV positive patients from west of Iran by ELISA and PCR,” Iranian Journal of Parasitology, vol. 9, no. 4, pp. 474–481, 2014.
[7] Q. Asgari, M. Fekri, and A. Monabati, ”Molecular genotyping of Toxoplasma gondii in human spontaneous aborted fetuses in Shiraz, Southern Iran,” Iranian Journal of Public Health, vol. 42, no. 6, pp. 620–625, 2013.
[8] Q. Asgari, J. Sarnevesht, M. Kalantari, S. J. A. Sadat, M. H. Motazedian, and B. Sarkari, ”Molecular survey of Toxoplasma gondii infection in sheep and goat from Fars province, Southern Iran,” Tropical Animal Health and Production, vol. 43, no. 2, pp. 389–392, 2011.
[9] A. Barazesh, B. Sarkari, F. Mehrabi Sisakht, S. Abdolahi Khabisi, R. Nikbakht, and M. R. Ravanbod, ”Seroprevalence and molecular evaluation of toxoplasmosis in patients undergoing chemotherapy for malignancies in the Bushehr Province, Southwest Iran,” Jundishapur Journal of Microbiology, vol. 9, no. 9, Article ID e35410, 2016.
[10] B. Sarkari, Q. Asgari, N. Bagherian et al., ”Molecular and serological evaluation of Toxoplasma gondii infection in reared turkeys in Fars Province, Iran,” Jundishapur Journal of Microbiology, vol. 7, no. 7, Article ID e11598, 2014.
[11] A. Daryani, S. Sarvi, M. Aarabi et al., ”Seroprevalence of Toxoplasma gondii in the Iranian general population: A systematic review and meta-analysis,” Acta Tropica, vol. 137, pp. 185–194, 2014.
[12] G. Singh and R. Sehgal, ”Transfusion-transmitted parasitic infections,” Asian Journal of Transfusion Science, vol. 4, no. 2, pp. 73–77, 2010.
[13] M. Foroutan-Rad, H. Majidiani, S. Dalvand et al., ”Toxoplasmosis in blood donors: a systematic review and meta-analysis,” Transfusion Medicine Reviews, vol. 30, no. 3, pp. 116–122, 2016.
[14] G. Karimi, A. Mardani, and M. Zadsar, ”Prevalence of Toxoplasma gondii among Iranian blood donors: a narrative review article,” Iranian Journal of Parasitology, vol. 11, no. 1, pp. 10–18, 2016.
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[15] L. Y. Norouzi, B. Sarkari, Q. Asgari, and S. A. Khabisi, "Molecular evaluation and seroprevalence of toxoplasmosis in pregnant women in Fars province, Southern Iran," *Annals of Medical and Health Sciences Research*, vol. 7, no. 1, pp. 16–19, 2017.

[16] H. Taghizadeh, R. Shahririarad, A. Erfani et al., "Seroepidemiological survey of toxoplasmosis among university students in Shiraz, southern Iran," *Annals of Tropical Medicine and Public Health*, vol. 10, no. 2, pp. 362–365, 2017.

[17] B. Sarkari, G. Hosseini, M. H. Motazedian, M. Fararouei, and A. Moshfie, "Prevalence and risk factors of intestinal protozoan infections: a population-based study in rural areas of Boyer-Ahmad district, Southwestern Iran," *BMJ Infectious Diseases*, vol. 16, no. 1, article 703, 2016.

[18] G. Hosseini, B. Sarkari, A. Moshfie, M. H. Motazedian, and S. Abdollahi Khabisi, "Epidemiology of human fascioliasis and intestinal helminthes in rural areas of Boyer-Ahmad Township, Southwest Iran; a population based study," *Iranian Journal of Public Health*, vol. 44, no. 2, pp. 1520–1525, 2015.

[19] R. Shafei, B. Sarkari, S. M. Sadjjadi, G. R. Mowlavi, and A. Moshfie, "Molecular and morphological characterization of Fasciola spp. isolated from different host species in a newly emerging focus of human fascioliasis in Iran," *Veterinary Medicine International*, vol. 2014, Article ID 405740, 10 pages, 2014.

[20] B. Sarkari, N. Ghabakhloo, A. A. Moshfiea, and O. Eilami, "Seroprevalence of human fascioliasis in a new-emerging focus of fascioliasis in Yasuj District, Southwest of Iran," *Iranian Journal of Parasitology*, vol. 7, no. 4, pp. 15–20, 2012.

[21] B. Sarkari, N. Pedram, M. Mohebali et al., "Seroepidemiological study of visceral leishmaniasis in Booyerahmad district, Southwest Islamic Republic of Iran," *Eastern Mediterranean Health Journal*, vol. 16, no. 11, pp. 1133–1136, 2010.

[22] A. Khosravani, B. Sarkari, H. Naghban, A. Sharifi, M. A. Toori, and O. Eilami, "Hepatitis B Infection among high risk population: a seroepidemiological survey in Southwest of Iran," *BMJ Infectious Diseases*, vol. 27, no. 12, article 378, 2012.

[23] B. Sarkari, O. Eilami, A. Khosravani, A. Sharifi, M. Tabatabaei, and M. Fararouei, "High prevalence of hepatitis C infection among high risk groups in Kohgiluyeh and Boyerahmad Province, Southwest Iran," *Archives of Iranian Medicine*, vol. 15, no. 5, pp. 271–274, 2012.

[24] B. Edvinsson, S. Jalal, C. E. Nord, B. S. Pedersen, and B. Evengård, "DNA extraction and PCR assays for detection of Toxoplasma gondii," *APMIS-Acta Pathologica, Microbiologica et Immunologica Scandinavica*, vol. 112, no. 6, pp. 342–348, 2004.

[25] A. Mansouri, M. R. Adhami Mojarad, G. Badfar et al., "Epidemiology of Toxoplasma gondii among blood donors in Iran: a systematic review and meta-analysis," *Transfusion and Apheresis Science*, vol. 56, no. 3, pp. 404–409, 2017.

[26] B. Eser and M. Yay, "Prevalence of anti-toxoplasma gondii antibodies in Turkish blood donors," *Ethiopian Medical Journal*, vol. 44, no. 3, pp. 257–261, 2006.

[27] V. Svobodová and I. Literák, "Prevalence of IgM and IgG antibodies to Toxoplasma gondii in blood donors in the Czech Republic," *European Journal of Epidemiology*, vol. 14, no. 8, pp. 803–805, 1998.

[28] R. S. Vaz, A. T. B. Guimarães, L. D. Bonanato, and V. Thomaz-Soccol, "Technical evaluation of serological screening tests for anti-Toxoplasma gondii antibodies to prevent unnecessary transfusion risks," *Revista Brasileira de Hematologia e Heminoterapia*, vol. 30, no. 4, pp. 277–280, 2008.

[29] S. M. Makki and A. H. Abdel-Tawab, "Anti-Toxoplasma gondii antibodies among volunteer blood donors in eastern Saudi Arabia," *Journal of the Egyptian Society of Parasitology*, vol. 40, no. 2, pp. 401–412, 2010.

[30] K. H. Zghair, B. N. Al-Qadhi, and S. H. Mahmoud, "The effect of toxoplasmosis on the level of some sex hormones in males blood donors in Baghdad," *Journal of Parasitic Diseases*, vol. 39, no. 3, pp. 393–400, 2015.

[31] H. M. Elsheikha, M. S. Azab, N. K. Abousamra, M. H. Rahbar, D. M. Elghannam, and D. Raafat, "Seroprevalence of and risk factors for Toxoplasma gondii antibodies among asymptomatic blood donors in Egypt," *Parasitology Research*, vol. 104, no. 6, pp. 1471–1476, 2009.

[32] P. Elhence, P. Agarwal, K. N. Prasad, and R. K. Chaudhary, "Seroprevalence of Toxoplasma gondii antibodies in North Indian blood donors: implications for transfusion transmissible toxoplasmosis," *Transfusion and Apheresis Science*, vol. 43, no. 1, pp. 37–40, 2010.

[33] A. J. C. Cook, R. E. Gilbert, W. Buffolano et al., "Sources of toxoplasmosis in pregnant women: European multicentre case-control study," *British Medical Journal*, vol. 321, no. 7254, pp. 142–147, 2000.

[34] A. Rostami, S. J. Seyedtaabaei, S. Aghamolai et al., "Seroprevalence and risk factors associated with toxoplasma gondii infection among rural communities in northern Iran," *Revista do Instituto de Medicina Tropical de São Paulo*, vol. 58, 2016.

[35] T.-Y. Chiang, M.-C. Kuo, C.-H. Chen et al., "Risk factors for acute toxoplasma gondii diseases in Taiwan: a population-based case-control study," *PLoS ONE*, vol. 9, no. 3, Article ID e90880, 2014.

[36] H. Mahmoudvand, E. S. Dezaki, S. Soleimani et al., "Seroprevalence and risk factors of Toxoplasma gondii infection among healthy blood donors in south-east of Iran," *Parasite Immunology*, vol. 37, no. 7, pp. 362–367, 2015.