

Seroepidemiological Study of Toxoplasmosis in Kavar District, Fars Province, Southern Iran: A Community-Based Survey

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Abstract

Background: Toxoplasmosis is a common zoonotic infection affecting both humans and animals. Its prevalence varies widely across regions depending on geographical, climatic, dietary, and social factors. This study aimed to assess the seroepidemiological status of toxoplasmosis among residents of Kavar district, Fars Province, southern Iran.

Methods: A total of 1,413 adults residing in Kavar district participated in this community-based survey. Venous blood samples (5 mL) were collected from each participant, and sera were tested for anti-*Toxoplasma gondii* antibodies using an in-house ELISA. All positive samples were subsequently retested with a commercial ELISA kit for confirmation. Data were analyzed using descriptive statistics and chi-square tests to examine associations between seropositivity and demographic or health-related variables.

Results: Of the participants, 708 (50.1%) were men and 705 (49.9%) were women. The largest age group was 46–55 years (n=579, 41%), while the smallest was 56–70 years (n=374, 26.5%). Anti-*Toxoplasma* antibodies were detected in 155 individuals, yielding a seroprevalence of 11%. No significant associations were observed between seropositivity and age, sex, occupation, or pre-existing health conditions. However, a significant correlation was found between toxoplasmosis and exposure to cats.

Conclusion: The study revealed a notable seroprevalence (11%) of toxoplasmosis among residents of Kavar district, which is lower than the national average reported in Iran. These findings highlight the need for targeted health education and preventive strategies, particularly regarding cat exposure, to reduce the risk of infection in this region.

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Introduction

Toxoplasmosis is a common parasitic infection caused by the intracellular single-celled organism *Toxoplasma gondii*. It is estimated that up to one-third of the world's population is infected with this parasite. Cats serve as the definitive host of *Toxoplasma*, while a wide range

of mammals, including humans, act as intermediate hosts.¹ Humans can become infected by consuming meat contaminated with cysts, as well as water or vegetables contaminated with the oocyst stage of the parasite.¹

The prevalence of toxoplasmosis varies widely across regions, reflecting differences in climate, cultural practices, hygiene standards, and the presence

of cats.^{1,2} In some areas, seroprevalence can be as high as 90%, while in others it may be as low as 10%. Despite its widespread occurrence, toxoplasmosis often goes unnoticed, as the infection is typically asymptomatic in immunocompetent individuals. However, it can cause severe disease in immunocompromised persons and in fetuses when primary infection occurs during pregnancy.³⁻⁵ Understanding the global prevalence and distribution of toxoplasmosis is crucial for developing effective strategies to prevent and control it.

Toxoplasmosis is a significant public health concern in Iran, as it is in many parts of the world. The seroprevalence of the disease in Iran varies widely across regions and populations, reflecting diverse environmental conditions, cultural practices, and demographic characteristics. Reported seroprevalence rates range from as low as 20% to as high as 70%.⁶⁻¹² These variations may be attributed to factors such as contact with cats, consumption of undercooked or raw meat, and hygiene practices. Understanding the seroprevalence of toxoplasmosis in various regions is crucial for developing effective public health strategies to prevent and control this infection.

Studies conducted in Fars Province, southern Iran, have shown that the prevalence of toxoplasmosis is significant and varies across different regions within the province.¹²⁻¹⁵ The region's diverse environmental conditions, cultural practices, and population characteristics contribute to these variations in seroprevalence.

The present study aimed to determine the serostatus of toxoplasmosis and examine its association with potential risk factors and underlying diseases, using data from a comprehensive Persian cohort in Kavar District, Fars Province. This extensive cohort study is being conducted in Kavar District by the Endocrine Research Center and the Digestive and Liver Research Center, both of which are affiliated with Shiraz University of Medical Sciences (SUMS).

Methods

Study Area and Sampling

The Kavar District, located 35 kilometers southeast of Shiraz, the capital city of Fars Province, has a population of approximately 71,856 residents. The district's geographical coordinates are 29°11'N latitude and 52°42'E longitude. The area is notable for its considerable population of stray cats and dogs, which frequently interact with humans as well as with goats and sheep. Agriculture, livestock farming, and cattle breeding constitute the primary economic activities of the region.

The Gastroenterohepatology and Endocrine Research Centers, both affiliated with Shiraz University of Medical Sciences (SUMS), are currently

conducting a comprehensive Persian cohort study in Kavar. At the start of this cohort, 4,997 individuals aged 35 to 70 years from Kavar City were enrolled. For the present cross-sectional study, a random sample of 1,413 individuals was selected from this cohort using SPSS software, with no exclusions applied.

The sample size was determined based on the reported prevalence of *Toxoplasma* infection in Iran, assuming a 95% confidence interval and a precision level of 0.02. The minimum required sample size was calculated as 915. To account for a potential dropout rate of 30%, the final recruitment target was increased to 1,413 participants.

A 5 mL blood sample was collected from each participant, and sera were stored at -20 °C until analysis. Demographic data and information on potential risk factors for toxoplasmosis were obtained from the cohort database.¹⁶

Ethical Consideration

The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences (Ref. No. IR.SUMS.MED.REC.1401.398).

Preparation of T. gondii Antigen

The RH strain of *T. gondii*, maintained in the Department of Parasitology and Mycology at Shiraz University of Medical Sciences, was used for antigen preparation. Approximately 6×10^6 tachyzoites in 0.5 mL of phosphate-buffered saline (PBS) were intraperitoneally injected into BALB/c mice. After 72 hours, proliferated parasites were aspirated from the peritoneal cavity with a syringe.

The collected fluid was centrifuged at 200 g for 4 minutes to sediment leukocytes and cellular debris. The supernatant containing *T. gondii* tachyzoites was transferred to a new tube and centrifuged again at 800 g to pellet the parasites. The sediment was washed twice with PBS. To separate parasites from one another, trypsin (0.1 g/mL) was added, and the suspension was gently mixed until the tachyzoites were completely dispersed.

The number of parasites was counted using a Neubauer chamber, and the suspension was adjusted to a concentration of 50×10^6 tachyzoites per milliliter. Parasite disruption was achieved through two freeze-thaw cycles followed by sonication. Sonication was performed at 50 watts for five cycles of 30 seconds each, with 20-second intervals between each cycle. The tachyzoites were examined microscopically to ensure complete lysis.

The antigen preparation was centrifuged at 1,300 g for 30 minutes at 4 °C. The supernatant containing parasite antigens was collected, and the protein concentration was determined. The final antigen

solution was stored at -20°C until use.

ELISA for the Detection of Anti-Toxoplasma Antibodies

Initially, the optimal concentrations of antigen, serum, and secondary antibody were determined using a checkerboard titration. For the ELISA assay, the *Toxoplasma* antigen was dissolved in a coating buffer (0.1 M carbonate/bicarbonate buffer, pH 9.6) at a concentration of $5\text{ }\mu\text{g/mL}$. Subsequently, $100\text{ }\mu\text{L}$ of this antigen solution was added to each well of a 96-well ELISA plate and incubated overnight at 4°C .

Following incubation, the antigen solution was discarded, and the wells were washed five times with PBST (PBS containing 0.05% Tween 20) using an ELISA washer. Blocking was performed by adding $100\text{ }\mu\text{L}$ of 3% non-fat skimmed milk in PBST to each well, followed by incubation at room temperature for 2 hours. After washing the wells as described previously, $100\text{ }\mu\text{L}$ of diluted serum samples (1:100) was added to each well, and the plate was incubated for 1 hour at room temperature.

The wells were washed again, and $100\text{ }\mu\text{L}$ of conjugated secondary antibody, diluted 1:3000, was added to each well. Plates were incubated for 1 hour at room temperature. After five additional washes, $100\text{ }\mu\text{L}$ of OPD substrate solution containing 3% hydrogen peroxide was added to each well, and the plate was incubated at room temperature for 15 minutes. The optical density (OD) was measured using an ELISA reader at 450 and 630 nm.

Data Analysis

The demographic information of the study participants and the ELISA test results were entered into SPSS software for analysis. The chi-square test was employed to examine the associations between *Toxoplasma* seropositivity and participants' demographic characteristics.

Results

In this study, 1,413 serum samples from residents of Kavar County were examined using the ELISA method to determine the seroprevalence of toxoplasmosis. Of the total participants, 708 (50.1%) were male and 705 (49.9%) were female. Regarding age distribution, 460 participants (32.6%) were aged 35–45 years, 579 (41%) were aged 46–55 years, and 374 (26.5%) were aged 56–70 years. Concerning exposure to cats, 4.1% of participants reported frequent contact, while 17.5% reported occasional contact.

Out of the 1,413 participants, 155 (11%) tested positive for *Toxoplasma* antibodies. Among the seropositive cases, 78 (11%) were male and 77 (10.9%) were female. Statistical analysis revealed no significant association between gender and toxoplasmosis seropositivity ($P>0.05$). The highest prevalence of seropositivity (11.7%) was observed in the 46–55-year age group, whereas the 35–45-year age group exhibited the lowest rate (10%). No significant association was found between age and toxoplasmosis seropositivity ($P=0.67$). Among the seropositive individuals, 30.2% reported either continuous or intermittent contact with cats, including 22.4% with frequent contact and 8.1% with occasional contact. Statistical analysis indicated a significant association between toxoplasmosis seropositivity and cat contact ($P=0.03$).

In this study, among individuals who tested positive for anti-*Toxoplasma* antibodies, the following percentages were observed: 10.8% were homemakers, 11.7% were retired or unemployed, 9.2% were mechanics and drivers, 8.3% were salespersons or self-employed, 5.1% were laborers, 3.1% were farmers and agricultural workers, and 6.7% were livestock owners. Statistical analysis indicated that the association between occupation and toxoplasmosis seropositivity was not significant ($P>0.05$).

Table 1: Characteristics of the study population and seroprevalence of toxoplasmosis in Kavar County, Fars Province, southern Iran

Characteristics		Frequency (Percent %)	Positive for anti- <i>Toxoplasma</i> antibodies (percent %)	P value*
Age (years)	35-45	460 (32.6)	46 (10)	0.671
	46-55	579 (41.0)	68 (11.7)	
	56	374 (26.5)	41 (11)	
Sex	Male	708 (50.1)	78 (11)	0.511
	Female	705 (49.9)	77 (10.9)	
Job	Housewife	660 (46.7)	71 (10.8)	0.867
	Retired	213 (15.1)	25 (11.7)	
	Driver	130 (9.2)	12 (9.2)	
	Employee	81 (5.7)	10 (12.3)	
	Freelance occupation	120 (8.5)	10 (8.3)	
	Labor	96 (6.8)	12 (12.5)	
	Farmer	98 (6.9)	14 (14.3)	
	Stockman	15 (1.1)	1 (6.7)	
Contact with cats	Continuous	58 (4.1)	13 (22.4)	0.003
	Occasionally	247 (17.5)	34 (13.8)	

Among all seropositive cases, 10.1% had underlying diabetes, 18.2% had epilepsy, 6.6% had neurological or psychiatric disorders, and 5.7% had learning disabilities. Statistical analysis revealed no significant association between toxoplasmosis seropositivity and diabetes, brain cancer, epilepsy, learning disabilities, or neurological and psychiatric disorders ($P>0.05$). Table 1 presents the characteristics of the study population and the prevalence of toxoplasmosis in Kavar County, Fars Province, southern Iran.

Discussion

Toxoplasmosis is widely recognized as a zoonotic infection affecting both humans and animals worldwide. The prevalence of the disease varies considerably across different regions, with relatively low rates of approximately 10% to 30% reported in North America and Southeast Asia, and moderate rates (30% to 50%) observed in Central and Southern Europe. The highest prevalence is generally found among populations living in warm and humid regions.^{1,2}

The prevalence of toxoplasmosis varies across different regions of Iran.^{4, 6-8, 14} A meta-analysis conducted in Iran reported an overall prevalence of *T. gondii* infection of 3.39%, with similar rates observed between men and women. The study also indicated a higher prevalence in the northern regions (Caspian Sea area). In contrast, mountainous and colder provinces such as Ardabil, as well as warmer provinces like Sistan and Baluchestan, exhibited lower prevalence rates.⁸ In the present study, the prevalence of toxoplasmosis among residents of Kavar County was 11%. This rate is lower than that reported in some other regions of Iran.¹⁷⁻¹⁹ Such differences in prevalence may be attributed to geographical and climatic conditions that influence the transmission of the parasite's oocysts.

Regarding age, the highest seroprevalence of toxoplasmosis in this study was observed in the 46–55-year age group (11.7%). This finding is consistent with a 2015 longevity study conducted in Jahrom, Fars Province, which reported the highest infection rate in individuals aged 40–50 years.²⁰ Several studies across different regions of Iran have demonstrated an increase in infection rates with advancing age, likely reflecting greater cumulative exposure to contaminated environments over time.^{6, 12}

In this study, the prevalence of infection among men (11%) was nearly equal to that among women (10.9%). Similar findings have been reported in other studies, such as Mehrbani et al. (2014) in Fars province, which also observed comparable seroprevalence of toxoplasmosis between men and women. This equal prevalence may be attributable to similar daily activities and behaviors among the two

genders, leading to comparable levels of exposure to contaminated environments.¹⁰

In the current study, a significant proportion of infections was observed among individuals who frequently had contact with cats. The statistical association between toxoplasmosis and cat contact is consistent with the established biology of *T. gondii*. Cats serve as the definitive host of this parasite and can shed oocysts in their feces, contaminating the environment and acting as a source of human infection. This finding underscores the importance of public health interventions aimed at preventing toxoplasmosis, including raising awareness about the risks associated with direct contact with cats and promoting effective hygiene practices. Nonetheless, it is essential to recognize that contact with cats is not the sole means of transmission for *T. gondii*. The parasite can also be acquired through the consumption of undercooked or raw meat from infected animals, as well as through the ingestion of oocysts present in contaminated water or soil. Further epidemiological studies are therefore needed to gain a more comprehensive understanding of the transmission dynamics of toxoplasmosis in this region and to identify additional risk factors for infection.²¹

Regarding underlying diseases among participants, the highest prevalence of infection was observed in individuals with epilepsy; however, no statistically significant association was found between epilepsy and *Toxoplasma* seropositivity.

Conclusion

This study provides valuable insights into the seroepidemiology of toxoplasmosis among residents of Kavar District, Fars Province, southern Iran. The overall seroprevalence rate of 11% suggests that *T. gondii* infection is present at a relatively low level compared to other regions worldwide. Such variation may reflect differences in geographical conditions, dietary habits, occupations, or other social factors. The significant association between seropositivity and cat contact underscores the need for targeted preventive measures, particularly reducing exposure to cat feces through improved hygiene and public awareness. Further research is required to identify additional risk factors and to inform comprehensive prevention strategies tailored to this population.

Authors' Contribution

BS and QA contributed to study conceptualization, design, and data analysis. MO, ER, and MSB performed the experiments and were responsible for data collection. AS contributed to the study design and data analysis. BS drafted the initial manuscript. All authors reviewed and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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