Original Article

Seroprevalence and detection of *Toxoplasma gondii* and *Echinococcus granulosus* in humans by indirect immunoglobulin G enzyme-linked immunosorbent assays in Baghdad

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ABSTRACT

The present study was conducted from December 2021 to April 2023 at the College of Health and Medical Technologies, Al-Bayan University, in Baghdad province, Iraq, to detect *Toxoplasma gondii* infection in humans by serological methods. Field studies were conducted using 1,500 sera samples from hospitals and private medical laboratories. The sera samples were separated and examined by indirect immunoglobulin G enzyme-linked immunosorbent assays to serologically detect *T. gondii* infection. The serological results demonstrated that 62% of cases were positive. The present study recorded a high rate of infection in both pregnant (70.4%) and non-pregnant women (53.6%). The serological results in males illustrated a rate of 57%, while the females showed an infection rate of 65.1%, demonstrating a non-significant difference. The highest and lowest rates of infection pertained to the 30-39 (90.2%) and < 20 (25.1%) age groups, respectively. Nevertheless, 7.8% of the 192 serologically tested sera samples were positive for *Echinococcus granuloses*. Regarding the age group, the rates of infection were 10.2%, 6.6%, and 14% in the 20-29, 30-39, and > 40 age groups, respectively, with a highly significant difference with *E. granuloses*. In terms of gender, the infection rate in males was 5.7%, while it was 10.2% in females, with a highly significant difference ($P \leq 0.01$). Finally, regarding the months, the peak infection rate was recorded in March.

Keywords: *Echinococcus granulosus*, Human, Serology, Seroprevalence, *Toxoplasma*

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1. Introduction

T. gondii is an intracellular protozoan infecting around one-third of the world's people. Humans and almost all warm-blooded animals are intermediate hosts of *T. gondii*, and cats play a crucial role in the epidemiology of *T. gondii* as the definitive host (1). Human infection generally occurs through congenital transmission, organ transplantation, food or drink contaminated with oocysts, and tissue cysts from undercooked meat (2). The dormant form of *T. gondii* mainly resides in nervous and muscle tissues in infected hosts, and until recently, latent infections in people were expected to be asymptomatic (3). Symptomatic infection is generally described as congenital infections acquired through the first trimester and more severe than those acquired in the second and third trimesters. Lymphadenopathy, reticular cell hyperplasia, and finally, ocular toxoplasmosis can be observed after congenital or acquired infection as a result of acute infection or reactivation (4). The tissue cyst-forming coccidium is a polygynous protozoan with a facultative heterogeneous life cycle. Human *T. gondii* infection is one of the most significant public health challenges, affecting one-third of the world's population (5). Especially immunocompromised people, such as HIV-positives, cancer patients, and organ transplant recipients. *T. gondii*, as an opportunistic protozoa, can lead to life-threatening consequences for patients. Moreover, after congenital infection, acute disease and ocular infection can occur (6, 7, 8). Cystic echinococcosis has international prevalence partially due to the capability of worms to adapt to the extensive variability of house and wild middle and final hosts (9, 10). This infection severely affects the health of humans and animals and has frequently been observed in countries in moderate regions, including parts of Africa, Australia, South America, Central Asia, and China (11, 12). In light of the aforementioned issues, the present study aimed to detect *T. gondii* and *E. granulosus* in humans and examine the seroprevalence of the parasite regarding gender, age group, and female gender in humans.

2. Materials and Methods

2.1. Human sample collection

This field study was conducted on 1,500 human blood samples from private clinics in different areas of Baghdad from December 2023 to April 2023. Blood samples were collected from humans (580 males and 920 females) aged between < 20 and ≥ 40 years. *E. granulosus* was passed out to 192 humans from different regions of Baghdad. Blood tests were collected from humans of both genders and different ages.

2.2. Serological technique

The human blood samples were disrupted in several wells (two for controls and more specimens) and plates in strip containers. Incubation was performed at room temperature (15-25°C) for 10 min; subsequently, 100 ul of enzyme conjugate was washed, enhanced, and stored at room temperature for 5 min. Following that, 100 ul of the chromogen was added to each well. It was mixed well by gently patting the lateral part of the band holder with the directory member for about 15 sec.

2.3. Statistical Analysis

The data were analyzed in SAS software (version 20.1). A two-way ANOVA and least significant differences (LSD) post hoc test were performed to assess significant differences among the means of the groups. The results were expressed as mean ± standard errors, and p-values of < 0.05 were considered statistically significant (13).

3. Results

A total of 1,500 human blood samples were registered as infected with *T. gondii*. The total rate of infection was 62% in human immunoglobulin G (IgG). Regarding the age groups, subjects younger than 20 recorded a 25.1% infection rate, while this rate was 88.5% among 20-29-year-old cases. The highest rate of infection was recorded as 90.2% in the 30-39-year-old subjects, followed by the ≥ 40-year age group (33.8%), with significant differences ($P \leq 0.01$) (Table 1). In terms of gender, females (16.2%) accounted for the highest infection rate, while the infection rate was 3.7% in males, with non-significant differences ($P > 0.01$) (Table 2). Finally, the present study recorded a high rate of infection in pregnant women (70.4%), with a lower rate in non-pregnant ones (53.6%) (Table 3). The total number of hydatidosis patients was 7.8% in this study (Table 4). In females, there were 9 infections (10.2%), compared to 6 infections (5.7%) in males (Table 5). These cases were in the age range of 20-40 years old. The most infected cases were in the age group of ≥ 40 years (14%), while the 30-39 age group had the lowest rate of infection (6.6%) (Table 6). Regarding gender, the highest infection rate was 10.2% in females, while the infection rate was 5.7% in males.

4. Discussion

It is crucial to understand the status of *Toxoplasma* infection in the general population, especially humans. This study estimated the seroprevalence of *T. gondii* infection rate in the Iraq population using the serological diagnosis. In this study, 62% of IgG seropositive cases were recorded. The results of this research were inconsistent with those carried out in neighboring countries, such as Pakistan and Qatar. The overall seroprevalence rates of infection were recorded at 29.45% and 29.8%, respectively (14, 15).

Table 1: Infection rate of *Toxoplasma gondi* in human according to ages groups by indirect ELISA IgG

Age	Examined No.	Positive No.	Percentage (%)
< 20yr	330	83	25.1
20yr-29yr	350	310	88.5
30yr-39yr	460	415	90.2
≥ 40yr	360	122	33.8
Total	1500	930	62
Chi-Square (χ^2)	---	---	72.414**
(P≤0.01).			

Table 2: Infection rate of *Toxoplasma gondi* in Human according to genders by indirect ELISA IgG

Gender	No. of examined samples	No. of positive samples	Percentage (%)
Males	580	331	57
Females	920	599	65.1
Total	1500	930	62
Chi-Square (χ^2)	---	---	72.282**
(P>0.01).			

Table 3: Seroprevalence of *Toxoplasma gondi* according to pregnant status by indirect ELISA IgG

Female status	No. of examined samples	No. of positive samples	Percentage (%)
Pregnant	747	526	70.4
Non-Pregnant	753	404	53.6
Total	1500	930	62
Chi-Square (χ^2)	---	---	8.5824**
(P≤0.01).			

Table 4: Total infection rate of *Echinococcus granuloses* in Human by using indirect IgG-ELISA

Host	No. of examined samples	No. of positive samples (%)	Percentage (%)
Human	192	15	7.8

Table 5: *Echinococcus granuloses* infection in human according to age group by using indirect IgG-ELISA

Age	Examined No.	Positive No.	Percentage (%)
< 20yr	48	0	0.00
20yr-29yr	49	5	10.2
30yr-39yr	45	3	6.6
≥ 40yr	50	7	14
Total	192	15	7.8
Chi-Square (χ^2)	---	---	11.802 **
** (P≤0.01).			

Table 6: *Echinococcus granuloses* infection rate in Human according to gender by using indirect IgG-ELISA

Gender	No. of examined samples	No. of positive Samples	Percentage (%)
Males	104	6	5.7
Females	88	9	10.2
Total	192	15	7.8
Chi-Square (χ^2)	---	----	7.155 **
** ($P \leq 0.01$).			

A related study in Iran demonstrated that the seroprevalence of *Toxoplasma* infection was 39%. At the same time, Latin America, Central and Eastern Europe, and Southeast Asia have a high seroprevalence of *T. gondii* infection 75%-85% (16, 17, 18). In another study in Mexico, the seroprevalence of *T. gondii* infection was reported as 20.26% (19). In China and India, seroprevalence estimations were 12.5% and 30.9%, respectively. Although some of these risk factors may have the lowest effect on the epidemiological condition, they can alter the epidemiologic pattern of infection in various regions, with geographical and climatic conditions being significant variables (20). The results of this research are in agreement with those obtained in another study in which the adult age group (≥45 years) had significantly higher serological rates (80%) than the younger one 20% (21). Our findings revealed that seroprevalence positive cases were predominantly detected in the older age group of ≥ 40 years (55%) than in the younger age groups of 0-19 years (29%) and 20-39 years (44%). Other studies reported similar results (22, 23). The reason for the increasing rate of serology in older age groups is unclear. It can be ascribed to their longer exposure to the risk factors, with increased contact with pets and consumption of contaminated food and water being two of the modes of transmission. The study revealed that the prevalence rate was lower in males (57%) than in females (65.1%). However, the two genders exhibited no significant difference in *Toxoplasma* serological tests. The results related to *Echinococcus* infection were in line with those of previous results demonstrating that the age range of 11-40 years had the highest rate of disease incidence (24, 25). In disagreement with another study who illustrated that the highest rate of infection pertained to the 20-30 age group (26). The seroprevalence of *E. granulosus* infection was obtained at 1.24% in an Uruguayan rural human (27). The seroprevalence of *E.* granulosus infection at 4.36% in South Kashmir, India, using an enzyme-linked immunosorbent assay (28).

In Iran, around 7.4% of cases were reported while the seroprevalence study on antibodies against hydatid cysts from Sarkari showed a rate of 7.2% (29, 30). This result can be attributed to the disruption experienced by this group of rural and other social workers, leading to increased exposure to sources of contamination. Clinically, cats are important reservoirs of zoonotic parasites. *T. gondii* and *Echinococcus* infections represent a considerable parasitic disease. The dearth of data in this regard highlights the necessity of research on molecular epidemiology and clinical pathology aspects of *T. gondii* and *E. granulosus* infection in Iraq. Controlling pets that serve as hosts for zoonotic parasites is essential to reduce the prevalence of the parasite. This involves limiting the number of pets and ensuring they receive veterinary treatment. *Toxoplasma* and *E. granulosus* are some of the most common parasites in developed countries and may cause chronic diseases.

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Authors' Contribution
Study concept and design: H. M.
Acquisition of data: S. A.
Statistical analysis: H.A.G.
Administrative, technical, and material support: Z.K.
Drafting of the manuscript: B.A.A.

Ethics
The study procedure was accepted by the ethics board of the, Al-Bayan University, Baghdad, Iraq.

Conflict of Interest
The authors declare that they have no conflict of interest in this article.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

References

1. Montoya J, Liesenfeld O. (2004). Toxoplasmosis Lancet 363, 1965–1976. CrossRef| PubMed| CAS| Web of Science Times Cited. 807.
2. Weiss, L. and Dubey, J. Toxoplasmosis: A history of clinical observations. *International journal for parasitology*. 2009.1:39(8):895-901.
3. Flegr J. Effects of *Toxoplasma* on human behavior. *Schizophrenia bulletin*. 2007:33(3):757-60.
4. Bhopale G. Pathogenesis of toxoplasmosis. Comparative immunology, microbiology and infectious diseases. 2003;26(4):213-22.
5. Tenter A, Heckeroth A, Weiss L. Toxoplasma gondii: from animals to humans. *International journal for parasitology*. 2000;30(12-13):1217-58.
6. Brinkman K, Debast S, Sauerwein R, Ooyman F, Hiel J, Raemaekers J. Toxoplasma retinitis/encephalitis 9 months after allogeneic bone marrow transplantation. Bone marrow transplantation. 1998;21(6):635-6.
7. Singh N, Gayowski T, Marino I. Toxoplasma gondii pneumonitis in a liver transplant recipient: implications for diagnosis. Liver Transplantation and Surgery. 1996;2(4):299-300.
8. Goebel W, Conway J, Faught P, Vakili S, Haut P. Disseminated toxoplasmosis resulting in graft failure in a cord blood stem cell transplant recipient. *Pediatric Blood and Cancer*. 2007;48(2):222-6.
9. Eckert J, Conraths FJ, Tackmann K. Echinococcosis: an emerging or re-emerging zoonosis. *International journal for parasitology*. 2000;*30*(12-13), 1283-1294.
10. Al-Shammary SQJ. Prevalence of *Echinococcus granulosus* in stray dogs and larval stage in human in Baghdad province. *Iraq J Vet Med*. 2002;18:105-111.
11. Šnábel V, Altintas N, D'amelio S, Nakao M, Romig T, Yolasigmaz A, Dubinský P. Cystic echinococcosis in Turkey: genetic variability and first record of the pig strain (G7) in the country. *Parasitology Research*. 2009;105,145-154.
12. Dakkak AJV. Echinococcosis/hydatidosis: a severe threat in Mediterranean countries. *Veterinary Parasitology*. 2010;174.1-2: 2-11.
13. Cary N. Statistical analysis system, User's guide. Statistical. Version 9. SAS. Inst. Inc. USA. 2012.
14. Abu-Madi M, Al-Molawi N, Behnke J. Seroprevalence and epidemiological correlates of Toxoplasma gondii infections among patients referred for hospital-based serological testing in Doha, Qatar. *Parasites & vectors*. 2008;1:1-9.
15. Tasawar Z, Aziz F, Lashari M, Shafi S, Ahmad M, Lal V, Hayat C. Seroprevalence of Human toxoplasmosis in southern Punjab, *Pakistan. Pak J Life Soc Sci*. 2012;1:10(1):48-52.
16. Bobić B, Jevremović I, Marinković J, Šibalić D, Djurković-Djaković O. Risk factors for Toxoplasma infection in a reproductive age female population in the area of Belgrade, Yugoslavia. European journal of epidemiology. 1998;14:605-10.
17. Song K, Shin J, Shin H, Nam H. Seroprevalence of toxoplasmosis in Korean pregnant women. *The Korean Journal of Parasitology*. 2005;43(2):69.
18. Ruiz-Fons F, Vicente J, Vidal D, Höfle U, Villanúa D, Gauss C, et al. Seroprevalence of six reproductive pathogens in European wild boar (Sus scrofa) from Spain: the effect on wild boar female reproductive performance. *Theriogenology*. 2006;1:65(4):731-43.
19. Galvan-Ramirez M, Troyo R, Roman S, Calvillo-Sanchez C, Bernal-Redondo R. A systematic review and meta-analysis of Toxoplasma gondii infection among the Mexican population. Parasites & vectors. 2012; 5(1):1-2.
20. Daryani A, Sarvi S, Aarabi M, Mizani A, Ahmadpour E, Shokri A, et al. Seroprevalence of Toxoplasma gondii in the Iranian general population: a systematic review and meta-analysis. Acta tropica. 2014;1:137:185-94.
21. Fan C, Hung C, Su K, Chiou H, Gil V, Ferreira M, Tseng L. Seroprevalence of *Toxoplasma gondii* infection among inhabitants in the Democratic Republic of Sao Tome and Principe. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2007;1;101(11):1157-8.
22. Shin D, Cha D, Hua Q, Cha G, Lee Y. Seroprevalence of Toxoplasma gondii infection and characteristics of seropositive patients in general hospitals in Daejeon, Korea. The Korean journal of parasitology. 2009;47(2):125.
23. Alvarado-Esquivel C, Estrada-Martínez S, Pizarro-Villalobos H, Arce-Quiñones M, Liesenfeld O, Dubey JP. Seroepidemiology of Toxoplasma gondii infection in general population in a northern Mexican city. The Journal of parasitology. 2011;1:97(1):40-3.
24. Al-Qadhi BN. *Study of some immunological and biochemical aspects of patients infected with hydatidosis*. Diss. Ph. D. Thesis. College of Science, University of Baghdad. 2005.
25. Werner A, Feliza K. An evaluation of diagnostic tests for hadatid disease. 1990;1:43-46.
26. Al-Jeboori T. Hydatid disease: a study of the records of the Medical City Hospital. *Journal of the Faculty of Medicine, Baghdad*, 1976;18(1/2):65-75.
27. Bonifacino R, Malgor R, Barbeito R, Balleste R, Rodriguez MJ, Botto C, Klug F. Seroprevalence of Echinococcus granulosus infection in a Uruguayan rural human

population. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1991;85(6):769-772.

28. Andrabi A, Tak H, Lone BA, Para BA. Seroprevalence of human cystic echinococcosis in South Kashmir, India. *Parasite Epidemiology and Control*. 2020;*11*, e00172.
29. Galeh TM, Spotin A, Mahami-Oskouei M, Carmena D, Rahimi MT, Barac A, Ahmadpour E. The seroprevalence rate and population genetic structure of human cystic echinococcosis in the Middle East: a systematic review and meta-analysis. *International Journal of Surgery*, 2018;51, 39-48.
30. Sarkari B, Sadjjadi SM, Beheshtian MM, Aghaee M, Sedaghat F. Human cystic Echinococcosis in Yasuj district in Southwest of Iran: an epidemiological study of seroprevalence and surgical cases over a ten-year period. *Zoonoses and public health*. 2010;*57*(2),146-150.