Original Research

SEROEPIDEMIOLOGY AND RISK FACTORS OF HUMAN TOXOPLASMOSIS IN ERBIL, KURDISTAN REGION, IRAQ

Dhary Alewy Almashhadany*[1], Abdulmunem Dherar Abdullah Aljoborey[2], Jiyan Ali Omar[2]

[1] *Department of Medical Laboratory Science, College of Science, Knowledge University, Erbil 44001, Iraq*

[2]*Department of Medical Microbiology, College of Science, Knowledge University, Erbil 44001, Iraq*

* Corresponding author: dhary.alewy@knu.edu.iq

ABSTRACT

Introduction: This study aimed at the detection of anti-*Toxoplasma. gondii* antibodies in human blood samples using immunological tests and to investigate the potential risk factors. **Methods:** Five hundred and twenty samples were collected from individuals aged between 10 to 71 years old. All samples were tested by latex, lateral flow assay, and Enzyme-linked immunosorbent assay (ELISA). Personal data which may be potential risk factors were collected. **Results**: Out of the 520 Blood samples tested, 23.3%, 25.4%, 20.2%, and 9.8% had positive results for latex, lateral flow assay, ELISA IgG, and ELISA IgM, respectively. The prevalence of *T. gondii* antibody was high in the elderly age group that had a seropositive result of 26.1%,27.5%, and 23.2% for latex, lateral flow assay, and ELISA IgG, respectively. **Conclusion**:The two major risk factors associated with the infection are contact with raw meats and consumption of raw vegetables.Latex and lateral flow assay for diagnosis of *T. gondii* in routine screening might be a cost-effective testing strategy. Consumers are recommended to heat the food of animal origin, and wash all vegetables and fruit carefully before consumption.

Keywords: Human toxoplasmosis, *T. gondii*, seroprevalence, risk factors

Introduction

Human toxoplasmosis, a global food-borne disease that is transmitted from animals, is a challenging infection caused by *Toxoplasma gondii*, an apicomplexan parasite that can infect any nucleated cell in any warm-blooded animal. This protozoan is a ubiquitous, single-celled, intracellular obligate parasite with a broad host range including humans, land and marine mammals, as well as different bird species (Manuel *et al*. 2020a; Chowdhury *et al*. 2021).

The epidemiology of *T. gondii* varies widely between countries and one-third of the human population is estimated to at risk of *T. gondii* infection. Indeed, toxoplasmosis has been reported from all regions worldwide, with seropositivity rates ranging from 0% to > 90% (Robert-Gangneux and Dardé 2012). Seroprevalence studies show a wide global variations, with as low as 4% in Korea to 78% in Nigeria (Montoya et al. 2014). Even within developed countries, it varies from 11% in Norway to 63% in Germany (Dama et al. 2016; Simon et al. 2020; Sroka et al. 2020). The parasite can persist for long periods in the human body, probably even for a lifetime. Most infections are asymptomatic because the immune system of healthy people normally restrains the protozoa from causing disease. However, pregnant women and immunosuppressed individuals may develop manifestations (McLeod et al. 2020; Tong et al. 2021).

T. gondii has a complex life cycle involving two sexual and asexual stages. In cats gut, replication of merozoites within enterocytes results in female and male gametes that are fused together toform the oocysts. The shedding of unsporulated oocysts with feces into the environment is followed by meiosis to produce haploid sporozoites in a sporulated oocyst. Oocysts can tolerate environmental conditions for log periods (Dubey et al. 2020). Sporulated oocysts containing sporozoites is the infective form that ingested by intermediate hosts (usually rodents) to initiate the asexual replication where the rapidly replicating tachyzoites are spread to nearly all body tissues. Sporulated oocysts can also contaminate food and water and reach diverse intermediate hosts. These intermediate hosts are usually become chronically infected after tachyzoites are differentiated into slow-growing bradyzoites in cysts. Sporozoites can also infect lifestock animals that transmmit the infecion to humans. Omnivorous or carnivorous animals (including humans) may also ingest tissue cysts and reinfect cats re-initiating the sexual phase of the life cycle (Yousefvand et al. 2021).

Humans usually become infected with *T. gondii* through uptake of inadequately prepared or raw meat or shellfish containing tissue cysts. Tachyzoites are also found in the milk of intermediate hosts. Contaminated food utensils or hands during food preparation can also lead to infection via the oral route (Mose et al. 2020; Marín-García et al. 2022). Fecal materials of infected kittens is an important source of *T. gondii* that contaminates foods and water (CDC 2018 Aug 19). Vertical transmission (from mother to child) is another important route that often leads to severe and lifelong disabilities in infected infants including brain calcification, hydro-encephalitis, vision disorders, and stillbirth or abortion (Damar Çakırca et al. 2023). Acquisition of toxoplasmosis after a blood transfusion or organ transplantation has also been documented (Manuel et al. 2020b; Rodrigues et al. 2020).

During the last two decades, several studies have revealed that latent *T. gondii* infection is associated with an increased risk of many neurological and diseases such as Alzheimer's disease, autism, bipolar disorder, schizophrenia, recurrent migraines, personality disorder, and even brain tumors (Yin et al. 2022). Moreover, infection complications may lead to cerebral and ocular damage and even death, especially in immunocompromised individuals (Greigert et al. 2019; Almeria and Dubey 2021). Furthermore, latent toxoplasmosis increases the risk for chronic heart failure, myocarditis, arrhythmia (Babekir et al. 2021; Zhou et al. 2021), liver cirrhosis (Babekir et al. 2022) , diabetes mellitus (Li et al. 2018; Asgari et al. 2021), and inflammatory bowel disease (Saraav et al. 2021).

The prevalence of toxoplasmosis in Iraq and the Kurdistan region is increasing and affecting all ages(Almashhadany and Mayass 2018; Yahya 2018; Almashhadany 2020a). Therefore, this work aimed to detection *T. gondii* in human blood samples of different age groups in both urban and rural areas of Erbil governorate, and determined potential risk factors.

Methods

Study Design And Sampling

Five hundred and twenty (520) blood samples (280 males and 240 females) were randomly collected from the individuals visiting Rezgary Hospital as well as other private medical facilities in Erbil governorate from July to December 2021. All participants in the study approved the involvement after they were informed about the study aims and the procedures, with confirmation that all data will be treated confidentially. The study was approved by the Ethics Committee of the General Directorate of Health in Erbil and according to the Helsinki Ethical Principles Declaration.

Five ml blood samples were collected in a vacutainer tube without anticoagulants. The tubes were labelled, kept in an icebox, and transported to the research centre laboratory at the College of Sciences (Knowledge University) for analysis (Almashhadany 2020b).

Personal Information

A structured questionnaire was used to collect personal information regarding suspected risk factors including gender, age, residence site, contact with raw meat, consumption of undercooked meat, consumption of raw vegetables, and contact with soil (Al Mashhadany 2020).

Latex Agglutination Test

Serum antibodies (IgM and IgG) against *T. gondii* were detected by the latex agglutination test kit (Plasmatec Laboratory Products, UK) according to the manufacturer's instructions. Briefly, a drop of serum was spread and mixed with 25 µl of the latex reagent over the test slide and tilted continuously for 4 minutes. Agglutination in the mixture indicates a positive result.

Lateral Flow Assay

Sera were tested for the existence of specific *T. gondii* antibodies (IgM and IgG) via an Immunochromatographic (IC) lateral flow cassette kit (Biozek- Medical Vissenstraat, Netherlands) according to the manufacturer's instructions. Briefly, a small drop of serum (approx. 20 µl) was introduced into the sample' well followed by 2 drops of buffer solution (approximately 80 µl). Within 15 -20 minutes, the results were recorded as positive if two colored lines appeared at T and C regions. The appearance of a single line in the C region is a negative result.

Enzyme-linked Immunosorbent Assay (ELISA)

The anti-*T. gondii* IgG and IgM antibodies were detected by a commercially available ELISA kit (Diagnostic Automation, Inc. China) which has been done according to manufactured company instructions. The antigen-coated micro-well plate was covered with diluted samples and then incubated at 37 ºC. After incubation, the wash buffer solution was used to remove unbound antibodies from the micro-well. The labeled (enzyme-conjugated) anti-human antibodies were added to the microwell plate and then incubated to allow complex formation with the immobilized antigen-antibody complexes. The microwell plate was washed to remove the unbound labelled antibodies. Substrates A and B were added and then incubated to produce a blue color indicating the presence of *T. gondii* antibodies in the sample. Color intensity was measured at 450 nm using a microplate reader.

Data were analyzed by SPSS, version 21. The normal approximation method was employed to calculate the confidence intervals. The alpha level was set to ≤ 0.05. The differences between groups were evaluated with a chi-square test. Results with *p*-value ≤ 0.05 was considered statistically significant.

Results

Seroprevalence of human toxoplasmosis

Out of the evaluated 520 blood samples, 23.3%, 25.4%, 20.2%, and 9.8% had positive results for latex, lateral flow assay, and ELISA, respectively. Only 9.8% of all individuals were IgM positive which indicates an acute infection. The estimated current infection among Erbil population is expected to be between 7.25% and 12.36% (95% CI).

Association of toxoplasmosis with age & gender

Seropositivity for *T. gondii* antibodies appeared to be highest among elderly people (aged 61 to 70 years). The status of current infection was significantly higher in adults than in adolescents and children (p = 0.008). No significant difference was detected between males and females in terms of seropositivity (p = 0.182) (Table 1).

Table 1: Seroprevalence of toxoplasmosis according to gender and age.

Characteristic	No. samples	Positive cases n (%)			
		Latex	Lateral flow assay	ELISA IgG	ELISA IgM
Gender					
Male	280	59 (21.1	65 (23.2)	54 (19.3)	23 (8.2)
Female	240	62 (25.8	67 (27.9)	51 (21.3)	28 (11.7)
Age					
≤ 10	59	10 (16.9)	12 (20.3)	9 (15.3)	1 (1.7)
11-20	71	14 (19.7)	15 (21.1)	12 (16.9)	3 (4.2)
21-40	129	31 (24)	34 (26.4)	26 (20.2)	14 (10.9)
41-60	130	32 (24.6)	35 (26.9)	28 (21.5)	17 (13.1)
≥ 61	131	34 (25.6)	36 (27.5)	30 (22.9)	16 (12.2)

Lifestyle-associated risk factors of human toxoplasmosis

The addressed potential risk factors for toxoplasmosis are summarized in (Table 2). Based on the latex test, residents of rural areas are significantly more seropositive ($p = 0.05$) than urban residents. However, the results of other tests showed insignificant differences. Additionally, consumption or contact with raw meat was associated with higher seropositivity ($p = 0.001$) according to latex, Lateral flow, and ELISA IgG tests (but not ELISA IgM). This indicates that raw meat is not the main route of the acquisition of *T. gondii* in Erbil. Similarly, contact with soil did not show any association with toxoplasmosis. In contrast, consumption of raw vegetables is significantly ($p = 0.03$) associated with an increase in seropositive acute cases (ELISA IgM test), indicating an important source of the infection.

Table 2. Associated risk factors for toxoplasmosis

	Positive cases n (%)			
	Latex	Lateral flow assay	ELISA IgG	ELISA IgM
Residence				
Urban	53 (43.8)	60 (45.4)	51 (48.6)	24 (47.1)
Rural	68 (56.2)	72 (54.6)	54 (51.4)	27 (52.9)
Contact with raw meat				
Yes	71 (58.7)	79 (59.8)	61 (58.1)	29 (56.9)
No	50 (41.3)	53 (40.2)	44 (41.9)	22 (43.1)
Consumption of undercooked meat				
Yes	71 (58.7)	79 (59.8)	61 (58.1)	29 (56.9)
No	50 (41.3)	53 (40.2)	44 (41.9)	22 (43.1)
Consumption of raw vegetables				
Yes	76 (62.8)	74 (56.1)	58 (55.2)	31 (60.8)
No	45 (37.2)	58 (43.9)	47 (44.8)	20 (39.2)
Contact with soil				
Yes	36 (29.8)	43 (32.6)	29 (27.6)	17 (33.3)
No	85 (70.2)	89 (67.4)	76 (72.4)	34 (66.7)

Discussion

Toxoplasmosis is one of the most significant protozoal diseases globally, predominantly in poor, tropical, and developing countries. Its epidemiology differs from one country to another, even from one area to another within the same country, in response to different eating habits and food processing practices among cultural groups in the same location (Saki et al. 2019; Yousefvand et al. 2021).

The prevalence of *T. gondii* found in the present study is consistent with previous studies in Erbil city (24.6%) (Al-daoody et al. 2019), Saudi Arabia (24.1%) (Aqeely et al. 2014), Palestine (17.6%) (Nijem and Al Amleh 2009), and Yemen (21.2%) (Al-Adhroey et al. 2019). However, higher prevalence rates were reported in Erbil (Iraq) (29.19%) (Al-Daoody 2012), Baghdad (40.65%) (Abdul-Hussein and Al-Marsomy 2020), Iran (33.8%) (Rostami et al. 2020), Duhok (Iraq) (35.61%) (Salih et al. 2020), and Malaysia (59.7%) (Wana et al. 2020). In contrast, lower seroprevalences were reported in different countries such as Zambia (5.9%) (Frimpong et al. 2017), Mexico (8.2%) (Alvarado-Esquivel et al. 2009), Sri Lanka (12.3%) (Chandrasena et al. 2016), and many European countries (Flegr et al. 2014).

The observed frequency of acute infection, inferred by IgM detection, in this study is slightly lower than recently reported by studies in Erbil city in 2017 (12.93%) (Abdullah and Mahmood 2017), and in 2019 (15.6%) (Akil Khudhair Al-Daoody et al. 2019). This difference may be attributed to differences in diagnostic tests employed and differences in sample size (263 and 167 vs 520 samples in our study). Generally speaking, prevalence rates can vary widely and depend largely on local environmental factors, the region of study, cooking habits, hygienic standards, socio-economic status, and method of detection (Flegr et al. 2014; Dama et al. 2016).

The observation of increased seropositivity for *T. gondii* in adult humans is consistent with the published literature. For instance, in Germany, the seroprevalence of human toxoplasmosis strongly increases from 20% (95% CI: 17–23%) in the 18–29 age group to 77% (95% CI: 73–81%) in the 70–79 age group (Wilking et al. 2016). Similar findings have also been reported recently from the United States where the seropositivity was strongly associated with age ($p < 0.0001$) increasing from 2.4% in the 18 to 29 years of age group to 22.1% in elderly people (60 to 85 years old) (Egorov et al. 2021). This could be explained by the fact that older people have a longer period of exposure to the risk factors of *T. gondii*.

Regarding potential risk factors, the residence area seems not to be a risk factor for the infection in Erbil, which is in good agreement with previously reported findings (Munoz-Zanzi et al. 2016; Tilahun et al. 2018) but contradicts other studies (Marques et al. 2008; Rivera et al. 2019). Here, we must pay attention to the fact that the positive association of the seroprevalence with the place of residence may reflect the lifestyle that makes the individuals more predisposed to the infection. The agricultural and farming activities in villages may expose people to contaminated water or soil. Moreover, handling animals, farming activities, and gardening are all crucial factors that contribute to a higher frequency in rural areas (Marques et al. 2008; Rivera et al. 2019).

The results also demonstrate that *T. gondii* infection was not significantly associated with contact or consumption of undercooked meat. Solid evidence supporting such observation is guaranteed throughout surveillance of active *T. gondii* infection among cattle, buffaloes, sheep, goats, and poultry in Erbil. However, our results are inconsistent with a study from Brazil that reported meat contact contributes greatly as a risk factor for the infection (83.6%) (Marques et al. 2008). However, the consumption of raw vegetables was found to be associated with the infection in this study. These findings are consistent with the results obtained from Norway (Kapperud et al. 1996), China (Cong et al. 2015), and France (Berger et al. 2009). The oocytes of *T. gondii* may be transferred to vegetables by contaminated water and soil. Despite the scarcity of data to confirm this, several epidemiological studies showed a strong association between eating unwashed raw vegetables and the primary infection (Bieńkowski et al. 2022; Khan et al. 2022).

According to the published literature, the highest priority should be given to prevent and reduce the risks of toxoplasmosis from food and the environment, which includes, avoidance of suspected raw meat, washing of vegetables and fruit carefully before eating, and proper cooking to ensure the destruction of infectious stages of *T gondii.* Additionally, hygiene during food preparation and after farming practices as well as water sanitation also contributes to decreasing infection rates. Careful washing of hands after caring for cats and feeding cats dried or canned food is also an efficient preventive measure. However, education about human toxoplasmosis and how to avoid it is strengthened by health professionals, principally as part of pre-and neo-natal awareness and with immunocompromised people. In any case, its efficiency depends on, the wholeness and precision of the advice being given, as well as how closely this is adhered to by patients (CDC 2018 Aug 19; Wana et al. 2020).

Conclusion

Toxoplasmosis is still one of the significant public health challenges for Iraqi Kurdistan. Rapid and reliable techniques such as Latex, Lateral flow cassette tests can be adopted for screening for infection, due to their easy methodology and affordable prices. The risk factors of human toxoplasmosis in Erbil Governorate are still not well understood. The dissemination of health awareness through media (audio, visual media, and newspapers) highlighting the mode of transmission of these protozoa is highly recommended. In addition to educating the community, especially in rural areas, with special emphasis on the risk of toxoplasmosis transmission via food or contact with a cat (or their litter) is an important factor to control *T. gondii* infections in humans.

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Conflicts of Interest

The author declares no conflicts of interest.

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