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Original paper

Seroprevalence and risk factors of toxoplasmosis among University of Kirkuk female students

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ABSTRACT. The study was conducted in the College of Education/Hawija, University of Kirkuk from September 1st, 2018 until the period February 28th, 2019. The first part included determine the prevalence of *Toxoplasma gondii*, which involved collecting blood from the female students from different stages in the different colleges of the University of Kirkuk, Kirkuk province (northeastern Iraq), ranged <20–24 years old. All necessary information was recorded using a questionnaire prepared for this purpose. In the current cross-sectional study, 210 blood specimens were collected from participants. Blood specimens were examined for evaluated the levels of specific anti-toxoplasma IgM and IgG antibodies using the protocol of an enzyme-linked immunosorbent assay (ELISA). The results revealed that the total infection of *Toxoplasma gondii* antibodies was 9.05% for IgG via 3.33% for IgM with significant difference at $p<0.05$. According to risk factors, the univariate logistic regression analysis revealed that only increase in domestic cats' owners and directly contact with the soil (gardening in the house) had a corresponding increase with distribution of the infection ($p<0.05$). Data in the present study revealed that the toxoplasmosis seroprevalence ratio among participants was 12.38%. It was observed that all of the risk factors in the present study had no statistically association with the toxoplasmosis seroprevalence, except cat ownership and house gardening once, where they were showed a highest odd ratio.

Keywords: *Toxoplasma gondii*, toxoplasmosis, seroprevalence, antibodies, risk factor

Introduction

Toxoplasmosis is a zoonotic disease; it is a worldwide distributed disease caused by *Toxoplasma gondii* (Coccidia) [1], where chronically infected about 30% of world population [2,3]. *T. gondii* is an obligate intracellular protozoan parasite responsible for common parasitic infections around the world in a wide range of warm-blooded animals including humans, and is considered one of the most successful eukaryotic pathogens [4–6]. The infection consequences via *T. gondii* may depend on certain factors such as parasite genotypes and host species [7]. In humans, the disease has varied indices, and it ranged from asymptomatic to severe acute toxoplasmosis [8,9]. Toxoplasmosis can cause serious complications in pregnant women, where in result leads to miscarriage, stillbirth, and birth defects [10], as well as it can cause rough neurologic, ocular toxoplasmosis, and systemic diseases in neonates and immunocompromised individuals [11–13].

Infection usually occurs orally either from oocysts shed by cats in water and on food, through tissue cysts in undercooked and/or raw contaminated meat [14,15], or trans-placentally. However, cats shed oocysts to the environment then sporulate and become infectious within 24 hours, depending on ecological conditions such as temperature and humidity. Sporulated oocysts can survive for months to years if existed in moist soil [15].

The immune system plays a major role in the prevention of toxoplasmosis through the innate immune mechanism and adaptive immune response. Toxoplasmosis stimulates two types of immune response; humoral and cell-mediated immune responses, the former associated with forming of active extracellular and invasive tachyzoite in the blood stream [16,17], antibodies are produced by stimulating B-lymphocytes (B-cells); known as IgM, IgG, IgA and IgE. These antibodies are functionally acting on the eradication of the infection and removing the parasite found free in body fluids by activation of supplement pathways and catalytic activity of immune system [18–20].

Therefore, in toxoplasmosis; the cellular immunity is the key element of the host's immune reaction, and human antibodies play a minor role but remain the important resources for diagnosing toxoplasmosis. Hence, the detection of specific IgM antibodies followed by specific IgG antibodies has been established for the diagnosis of acquired toxoplasmosis [21,22].

For detection of *T. gondii*-specific immunoglobulin, recently, several sero-techniques have been applied. However, among different types of the serological diagnostic techniques, Enzyme-Linked Immunosorbent Assay ELISA is considered one of the easiest diagnostic tests to be depended [23–25].

The present study attempts to detection the presence of specific antibodies against *T. gondii*, and to recognize the risk factors and probable contamination means among University of Kirkuk female student in Kirkuk Province-Iraq to draw attention to this abandoned disease.

Materials and Methods

Study area and participants

The present cross-sectional study was established in Kirkuk city (Latitude: 35°47′05″N; Longitude: 44°39′3″E; Elevation above sea level: 350–400 m; population: about 1.75 million) from September 1st, 2018 to May 31st, 2019. The study was conducted on 210 speciously healthy female students of different faculties of Kirkuk University.

Data collection

Data was collected using a structured questionnaire which was given to each healthy participant who subjected to the current study. The questionnaire composed questions related with demographic clinical data and some other information including 'ABO' blood grouping, pregnancy history, age, knowledge on toxoplasmosis, marital status, socioeconomic status and standard of living. Also, information on potential risk factors which may related with prevalence of toxoplasmosis such as eating behavior, kitchen hygiene, and history of blood transfusion, owning cats, direct contact or handling of domestic cats, direct contact with cat boxes and/or soil, eating raw or undercooked meat and improperly washed vegetables or fruits.

Sample collection

Aseptically, 5 ml of venous blood was collected "using suitable vacuumed blood collection system" from 210 healthful female students aged <20–24 years old in different colleges of the University of Kirkuk after a written informed was obtained from each participant before collection of samples. The blood specimens were then labeled and transported directly to the Ibn El-Nafis private medical Laboratory-Kirkuk province in the cool box, as sequential batches, until further analysis.

Blood groups and types

To detection the blood groups and types, each obtained blood specimens has subjected to ABO blood grouping and blood type test. The Anti-A and Anti-B blend test have been carried out according to the manufacturer's protocol. Simultaneously, Anti-D blend method was applied, where a slide test depended which was based on agglutination method for Rhesus typing 'Rh' in serum; using monoclonal/polyclonal blend reagent.

Laboratory processing

Serum was obtained from the whole blood by centrifugation at 3000 rpm for ten minutes at room temperature. The separated serum was transferred into Eppendorf tubes, labeled and kept at −20°C until further use. Each serum specimen was examined for anti-*T. gondii* IgG and IgM antibodies using Enzyme Linked Immunosorbent Assay (ELISA) test kit (Human Gesselschaft for Biochemical and Diagnostic, Germany) according to the manufacturer's instruction [26,27]

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Data analysis

The obtained data were analyzed by IBM SPSS Statistics software package (version 22, USA). Pearson's Chi-squared/Fisher's exact tests were used to compare the sero-prevalence values, related to the characteristics of the subjects. To identify the significant variables; primarily, a univariate logistic regression was then performed, for use in a multivariate logistic regression analysis. The odds ratios (ORs) with their 95% Confidence Interval (CI) were employed for various risk factors associated with seropositivity were estimated. Probability (p-values) of <0.05 were considered as the level of statistically significance.

Results

Concerning to the toxoplasmosis knowledge among the participants; the obtained data of questionnaire revealed that 48.66% of the

Table 1. Seroprevalence of anti-*Toxoplasma gondii* IgG and IgM antibodies among the female students

Antibodies	Seropositive (%)	Seronegative (%)	Total
IgG	19 (09.05)	191 (90.95)	210 (100)
IgM	07 (03.33)	203 (96.67)	210 (100)
Pearson[1] χ^2	5.219	0.189	00
p-value	0.026*	0.674	00

1: Fisher's exact tests

participants have never seen information about toxoplasmosis, prior to the interview, and 69.89% of the participants were oblivious to being serologically examined or not for toxoplasmosis.

Two hundred and ten blood specimens were collected from healthful female of selected students aged <20–24 year from different colleges of Kirkuk University which were subjected to the present study. The most participants were aged <20 years old (84/210;40%). All serum specimens have been examined for IgM and IgG antibodies using ELISA protocol. The obtained results are summarized in Table 1 and 2.

All collected blood specimens were investigated for anti-*T. gondii* IgG and IgM. The overall seroprevalence of *T. gondii* was 26/210 (12.38%); 19/210 (09.00%, 95% CI=5.55–13.6) of students had chronic toxoplasmosis categorized by the incidence positive IgG antibodies, 07/210 (03.33%, 95% CI=1.08–7.73) of students had acute categorized by the incidence positive IgM antibodies. It is noteworthy that; of the 7 students with IgM titers, two also had IgG titers. Only two out of 7 students had sub-acute toxoplasmosis categorized by the incidence positive of both IgM and IgG antibodies with statistically no significant differences ($p>0.05$), Table1.

Data in Table 2 shows that among 210 participants; the highest seroprevalence *Toxoplasma*-IgG was recorded to be 47.40% in the age group of (20–22) years, the highest seroprevalence of *Toxoplasma*-IgM was noticed among the age group of (22–24) years which was 42.86%, with no significant differences ($p>0.05$).

Regarding to the blood groups; no significant differences were observed between the groups, despite the blood group "O" shows the highest ratio of *Toxoplasma*-IgG and *Toxoplasma*-IgM; as 8/19 (42.1%) and 4/7 (57.1%), respectively ($p>0.05$). This finding was in contrary to the AB group results which were 15.8% and 0.00% for both of *Toxoplasma*-IgG and *Toxoplasma*-IgM, respectively ($p>0.05$).

Concerning abortion cases and due to the pregnant participant questionnaire answers, it observed that 19/210 (9.05%) of pregnant participant had an abortion history via 191/210 (90.95%) of non-pregnant had not ($p<0.05$) However, there was a no significant difference between seropositivity among participants of urban and rural inhabitants ($p>0.05$). Regarding to the marital status; the highest seroprevalence of chronic and acute toxoplasmosis infections with no significant differences was recorded among bachelor and married female students, where were 11/19 (57.9%) and 4/7 (57.1%) ($p>0.05$), respectively.

Data in Table 2 also indicates that 16/210 (7.62%) of the participants own domestic cats via 194/210 (92.38%) of students who did not own domestic cats. The cat's owner variable was showed significant relationship ($p<0.05$) with chronic and acute infections, data has been recorded as 5/19 (26.30%) and 4/7 (57.10%), respectively. There was no significant difference associated with meat consumption variable, although the highest seroprevalence of chronic toxoplasmosis was recorded among cooked meat consumptions 18/210 (94.70%).

About 15% of raw or unwashed fruit and vegetables consumers was recorded; with chronic and acute seropositivity of 5/19 (26.30%) and 1/7 (14.30%), respectively with no significant difference. However, the statistical analysis showed no significant differences ($p<0.05$) between female students who's dealing directly with the soil (gardening in the house), where were 8/19 (42.10 %) and 1/7 (14.30%) of each chronic and acute *Toxoplasma*-incidence, respectively; as well, concerning the female students having fast-food consumption behavior "eating outdoor non home

Table 2. Characteristics features of the subjected participants

	Groups	Categories (%) (n=210)	Anti-toxoplasmosis seropositivity (%) IgG$^+$	IgM$^+$	Pearson^1 χ^2	*p*-value
Age groups (years)	< 20	84 (40.00)	7 (36.80)	1 (14.28)	2.492	0.288
	20–22	67 (31.90)	9 (47.40)	3 (42.86)		
	22–24	59 (28.10)	3 (15.80)	3 (42.86)		
Blood groups	A	55 (26.19)	2 (10.50)	1 (14.30)	1.434	0.698
	B	49 (23.33)	6 (31.60)	2 (28.60)		
	AB	39 (18.57)	3 (15.80)	0 (0.00)		
	O	67 (31.91)	8 (42.10)	4 (57.10)		
Abortions	pregnant	19 (09.05)	13 (68.40)	6 (85.70)	0.778	0.629
	Non-pregnant	191 (90.95)	6 (31.60)	1 (14.30)		
Residency	Urban area	154 (73.33)	12 (63.20)	6 (85.70)	1.222	0.375
	Rural area	56 (26.67)	7 (36.80)	1 (14.30)		
Marital status	Married	39 (18.57)	7 (36.80)	4 (57.10)	1.919	0.383
	Divorced	5 (02.38)	1 (05.30)	1 (14.30)		
	Bachelor	166 (79.05)	11 (57.90)	2 (28.60)		
Cats ownership	Yes	16 (07.62)	7 (36.84)	6 (85.71)	4.887	0.037*
	No	194 (92.38)	12 (63.16)	1 (14.29)		
Meat consumption	Rawness	5 (02.38)	1 (5.30)	2 (28.60)	2.723	0.167
	Cooked	205 (97.62)	18 (94.70)	5 (71.40)		
Fruit or vegetables consumption	Raw/unwashed	31 (14.76)	5 (26.30)	1 (14.30)	0.417	0.471
	Washed	179 (85.24)	14 (73.70)	6 (85.70)		
Contact with soil (Gardening)	Yes	31 (14.76)	15 (78.95)	2 (28.57)	5.736	0.028*
	No	179 (85.24)	4 (21.05)	5 (71.43)		
Fast food	Yes	86 (40.95)	9 (47.40)	2 (28.60)	0.74	0.658
	No	124 (59.05)	10 (52.60)	5 (71.40)		

Data represented as No. (%); 1: (Fisher's exact tests); IgG$^+$ (n=19); IgM$^+$ (n=7)

cooked meals", were showed statistically non-significant differences (p>0.05).

Data in Table 3 illustrates the results of the univariate and multivariate logistic regression analyses for potential risk factors of *Toxoplasma* IgG among the participant. Univariate logistic regression analysis revealed that only increase in domestic cats' owners (OR=5.84, 95% CI=1.78–19.187, p=0.004) and female students whom directly contact with the soil (gardening in the house) (OR=5.31, 95% CI=1.94–14.58, p=0.001) had a corresponding increase with distribution of the infection. Other variables such as; eating outside of the home behavior "fast food", Age and consumption raw or

Table 3. Anti-toxoplasmosis seropositivity and risk factor

	Group	Univariate analysis			Multivariate analysis		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age (years)	<20	1.65	0.48–5.63	0.424	1.015	0.95–1.09	0.67
	20–22	1.35	0.36–5.05	0.653	1.005	0.94–1.08	0.89
	22–24	1	ref.	–	1	ref.	–
Blood groups	A	0.278	0.057–1.37	0.116	0.988	0.905–1.079	0.79
	B	1.029	0.33–3.18	0.96	0.982	0.899–1.073	0.69
	AB	0.615	0.153–2.47	0.492	0.972	0.886–1.066	0.541
	O	1	ref.	–	1	ref.	–
Residency	Urban area	0.59	0.22–1.59	0.297	1.021	0.97–1.07	0.42
	Rural area	1	ref.	–	1	ref.	–
Cats ownership	Yes	5.84	1.78–19.187	0.004*	4.98	1.38–10.85	0.017*
	No	1	ref.	–	1	ref.	–
Meat consumption	Rawness	2.6	0.28–24.5	0.404	2.11	1.09–6.89	0.301
	Cooked	1	ref.	–	1	ref.	–
Fruit or vegetables consumption	Raw/ unwashed	0.44	0.15–1.33	0.145	1.58	0.85–6.64	0.211
	Washed	1	ref.		1	ref.	–
Contact with soil (Gardening)	Yes	5.31	1.94–14.58	0.001*	4.96	1.24–11.56	0.023*
	No	1	ref.	–	1	ref.	–
Fast food	Yes	1.33	0.52–3.43	0.552	0.76	0.43–2.46	0.512
	No	1	ref.	–	1	ref.	–

*p<0.05; (OR): Odd Ratio; (CI): Confidence interval; ref.: reference; 1: Fisher's exact tests; χ^2= 6.253, p-value=0.66

undercooked minced meat were showed moderately increased odds ratios (1.33, 1.35, 1.65 and 2.6) of infection, respectively, but without significant differences (p>0.05 for all). According to the multivariate logistic regression analysis; it was further suggested approximately the same results of univariate logistic regression analysis.

Discussion

Toxoplasmosis is a global, widely distributed protozoan disease of great medical importance, caused by Apicomplexan parasite *Toxoplasma gondii*. The questionaries' results in the present study exhibited the lowest level of overall knowledge about the toxoplasmosis symptoms, transmission mechanisms and prevention measures among subjected female students. The highest level of knowledge among the students in the current study was that the toxoplasmosis might hurt pregnant women, and the infection might transmit to the fetus and/or have serious consequences on it.

The obtained data in the present study revealed that overall prevalence of toxoplasmosis among participants was 12.38%; 9.05% were seropositive for chronic toxoplasmosis and 3.33% were seropositive for both acute and sub-acute toxoplasmosis which are less than the previous

studies conducted in various regions in Iraq; 32.24%, 19.14%, 21.5% and 21.94% of. students were positive for anti-*Toxoplasma,* antibodies in secondary female students, as well as in different, colleges of Kirkuk University [28,29], Najaf [22] and Thi-Qar University [30], respectively, and at variance to the result of the study conducted in College of Medicine and College of Pharmacy, University of Basra illustrated that the overall seropositive for *Toxoplasma* was 12.43% [31]. The latter is agreeing with the current study result. The obtained data in the present study were less than the seroprevalence already found in different neighboring countries in and/or close to the Middle East, including Saudi Arabia 9.4%, Jordan 47.1%, Iran 75.7%, Yemen 45.4% [19,32–34], and Ethiopia 85.4%, Malaysia 13% and Brazil 24.1% [16,35,36].

It is noteworthy that there are variety between the seropositivity rates of the current study and those in prior studies. The variations in the seropositivity rates may be due to several factors, for instance; age groups of the participating in these studies, local nutritional habits, climate, personal hygiene manners, the socio-economic status and geographic location [37–41]. Otherwise, the lower rates of the infection among the participants in the present study could be as a result of higher education, as shown to be a decreasing factor in *T. gondii* infection. Another reason for a lower prevalence rate of toxoplasmosis among the female students in the current study might be the lower age of the participants and/or education grade which subsequently in turn lowered the exposure to *T. gondii* and the infection **[**42,43].

The associations of age and toxoplasmosis seroprevalence indicated that seropositivity started at age group of <20, and increased between participants gradually of 20 years through 22 years of age; then the rate was slightly decreased among the subsequent age groups. Statistically, there was no significant variance between age group associations with toxoplasmosis seroprevalence. Despite previous studies indicated that the prevalence of the infection is associated with the age, where some authors noticed that the highest prevalence rate among women aged ≥ 36 years [44–46], but others recorded the higher seroprevalence rate among pregnant women aged 20–30 years [47–50], the latter studies were stated that the infection rate has increased among women between 20–30 years old; these results are much closer to that results in the current study.

The finding results in the present study demonstrate the association between blood group system and toxoplasmosis infection, where the highest infection was among O blood group (42.10% and 57.10%), followed by B blood group (31.60% and 28.60%) for each chronic and acute *Toxoplasma*, respectively. Hence, it supports the studies what suggest that the B antigen, represented in B and AB blood groups, acts as a probable receptor for *T. gondii* in the gastro-intestinal tract [51,52]. The mechanism of microorganism's adhesion to the host's mucous membranes is not absolutely clear, but it seems that the ABO group systems glyco-conjugates are involved in this process [53]. The finding in the present study is agree with the studies [54–56].

In the current study, the data showed that the seropositive toxoplasmosis frequency among aborted pregnant students by using ELISA test was found to be 9.05%. The seroprevalence rates of aborted pregnant participants of each IgG and IgM were 68.4% and 85.7%, respectively; while the anti-toxoplasma antibodies were IgG 31.6% and IgM 14.3% in non-pregnant participants, these results are not match and lower than with those in [57], where they determined the incidence of *T. gondii* in aborted women who attended Al-Basrah Hospital for women and children in Al-Basrah province, Iraq, and they recorded the positive result of antibodies IgG of 73% and 33% was positive samples of antibodies, using ELISA technique.

A contrary result was observed through reviewing the literatures, where a study carried out in Durango city, Mexico, as their results; IgG and IgM antibodies rates found to be 7.2% and 31%, respectively [58]. Nevertheless, the findings in the current study agree with recent studies that were conducted in Iraq, provinces of Erbil, Baghdad, Tikrit (Samaraa city), Garmian district (Kurdistan Region), Duhok and Najaf [59–64], as well as a similar result was obtained by [65] demonstrated that the *Toxoplasma*-seropositive of IgM and IgG among pregnant women in Zanjan, Northwest of Iran were positive in 1.4% and 37.2% respectively. Moreover, the findings in this study compatible with those researches results that were carried out in Qatar, Saudi Arabia and Iran using ELISA protocol, where they found that the anti-*T. gondii* IgG and IgM had to be 35.1% and 5.2% [66]; 29% and 3% [67]; 27% and 3.33% among investigated women, respectively [68].

Elevated seroprevalence rates were recorded, for

instance; in France, authors reported that the seroprevalence was 58.2% [69], and others pointed that the toxoplasmosis seroprevalence in France decreased gradually among pregnant women, from 54.3% in 1995 to 43.8% in 2003 [70]. However, the variations in above mentioned results may be attribute to size of collected data or genetic and ethnic background of the study population, as mentioned by [71].

Regarding to the residence factor, the toxoplasmosis distribution showed no significant differences. However, the higher percent of toxoplasmosis in the study groups was (73.3%) in the urban site comparison with the rural ones. These findings are agreeing with results of [72] in Waset province, as well as in Najaf province, Iraq which confirmed by [64], similar results are noticed in Yemen among precipitants in urban region [73]. Conversely, studies were found in the literatures with inverted results, for instance; in Baghdad as mentioned by [60]. The findings of the current study may be attributed to the dealing with high number of cases in urban area comparing with those in the rural ones and their different habits in consumption of fast foods which may be a major infection source of *T. gondii*. Furthermore, according to the pathological history which obtained through the questionnaire in the current study, a considerable percentage of female students in Kirkuk province (>30%) were residents in rural areas, and/or recently, since the demographic modification policy which occurred in Kirkuk city, the immigration from the rural to the urban side and resident there may be the reason for increasing in the toxoplasmosis seroprevalence through the city.

Concerning of marital status factor and its distribution association with *T. gondii* antibodies seropositivity, the highest seropositivity IgG and IgM antibodies rate in the present study was found in the married female students with no significant differences ($p>0.05$), where were 36.80% and 57.10%, respectively. These findings agreeing with the study has been conducted in Saudi Arabia and France [32,74].

A statistical association have been noticed between cat ownership risk factor and the toxoplasmosis seropositivity. Where the highest rate of IgM antibodies seropositivity was recorded among cat owners' students, where was 85.7%, while the highest IgG antibodies rate was 63.16% among students whom have not own cats ($p<0.05$). As well as the direct contact with soil "gardening" factor showed the same toxoplasmosis seroprevalence association with a high statistical difference as it is obvious in the Table 2, harmonic results were found in studies conducted in Ethiopia [75,76]. However, the findings in the current study were contrary to [73] they noticed that the toxoplasmosis seroprevalence correlated with presence of cats were 28.4%.

Other risk factors in the present study as shown in Table 2 such as meat, fruit/vegetables and fast-food consumptions were not found to have any significant association with the seroprevalence of toxoplasmosis ($p>0.05$).

The distribution of the toxoplasmosis in the present study may be attributed to the direct contact with cats and/or frequent exposing to the sporulated oocysts by ingestion of these oocysts through farming the contaminated soil with cat feces, or eating contaminated under cooked meat with the oocysts [77].

According to other studies conducted in Egypt, Mexico, Norway, European research network and United States, reported that there are associations between distribution of toxoplasmosis and direct contact with soil [78,79], cat faeces cleaning [80] indoor or outdoor cat housing [79], ingestion of raw meat [81] and gardening [82]. These mentioned factors were assessed in the current study; however, no associations were found, except contact with soil and gardening.

Among the different risk factors statistically analyzed in the present study; as shown in Table 3, only cat ownership and direct soil contact "gardening" factors were correlated with toxoplasmosis positively, and were showed the highest odd ratios ($p<0.05$) comparison with the other factors. These factors were also identified significantly by other analysts [83,84]. Nevertheless, some studies did not find any correlation between toxoplasmosis and the contact with the soil, as described by [42,85].

The association of human toxoplasmosis and cats is difficult to assess by epidemiological studies since the soil is the main accused source, but not the cats. However, *Toxoplasma* oocysts cannot be found on the cat fur [86] and are often buried in soil along with cat faeces, and direct soil contact is universal and difficult to avoid. Sporulated oocysts can remain infective for 12 to 24 months in the moist soil, temperature, favorable shady location and poor sanitation [87]. Hence, it is necessary to increase awareness of how *Toxoplasma* infections occur so

women can find a suitable way to avoid the incidence of this serious parasitic infections.

In conclusions, the seroprevalence of *T. gondii* antibodies observed in the present study was relatively low among the sample of female university students in University of Kirkuk, Iraq. The direct contact with soil "gardening" and cat's ownership were identified as independent risk factors for *T. gondii* infection. The obtained results highlight the necessity to raise awareness of toxoplasmosis, specifically with regard to the way infections occur so that women can take serious steps to protect themselves and avoid contracting this parasitic infection. Moreover, studies on a wide scale are required to support future public health strategies.

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