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RESEARCH ARTICLE

TOXOPLASMA GONDII INFECTION AMONG PREGNANT WOMEN IN MUKALLA CITY, HADHRAMOUT, YEMEN: FACTORS ASSOCIATED WITH SEROPREVALENCE

Eidha Bin-Hameed[1,2,*], Omniya Bawzeer[1], Jawaher Al-Muhtdi[1], Rania Basreeh[1], Soumaya Alyazedi[1], Fatma Bahah[1], Nada Ba yazed[1], and Warda Bamerdah[1]

[1] *Dept. of Biology, Faculty of Science, Hadhramout University, Yemen*

[2] *Dept. of Health Sciences, Faculty of Medicine and Health Sciences, University of Science and Technology, Aden, Yemen*

***Corresponding author: Eidha Bin-Hameed; E-mail: e.binhamiad@hu.edu.ye &e.binhameed@ust.edu**

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Abstract

Toxoplasmosis is one of the protozoan diseases caused by *Toxoplasma gondii* (*T. gondii*) which infects a wide variety of hosts, including human population with cats being the definitive host. Congenital transmission can occur, which may result in fetal, neonatal death, or severe malformations. In this study, we aimed to determine the seroprevalence and associated risk factors of *T. gondii* infection among pregnant women in Mukalla city/Hadhramout, Yemen. We conducted cross-sectional and analytical study from March to June 2022 in Mukalla city, Hadhramout. Pregnant women aged 13 to 40 years old attending the gynecological and obstetric clinics were enrolled in the study. For each participant, socio-demographic, predisposing factors, and clinical data were collected through a questionnaire. Serological test (immunochromatographic assay) was performed for the detection and avidity determination antibodies of the latent toxoplasmosis (IgG) and active toxoplasmosis (IgM). Logistic regression analysis was used to identify factors associated with seropositivity. Out of 104 participants, 46.2% were positive for specific anti-*T. gondii* IgG antibodies and 10.6% had IgM antibodies suggestive of recent primary infection. The participants aged 20 to 30 years showed the highest IgG and IgM seroprevalence. The univariate logistic regression analysis showed that eating raw foods (OR = 4.449, CI: 1.311-15.093, p = 0.017), being often in contact with cats (OR = 3.929, CI: 1.143-13.502, p = 0.030) and being often in contact with cat feces (OR = 2.858, CI: 0.967-8.449, p = 0.058) were significantly associated with seropositivity. Multivariate analysis confirmed that eating raw foods (Adjusted OR = 4.428, CI: 1.249-15.698, p = 0.021) was significant predictor factor of *T. gondii* seroprevalence among the studied participants. Our findings suggest that *T. gondii* is widespread in the local area, resulting in a high exposure risk of pregnant women. Occupational predisposing factors appear to play a significant role in the transmission of toxoplasmosis. To avoid the potentially serious consequences to the fetus, there is an urgent need for systematic screening during antenatal care and the gynecological clinics visits and awareness health campaigns.

Keywords: *Toxoplasma gondii*; Seroprevalence; Pregnant women; Pregnancy; Risk factors.

Introduction

Toxoplasmosis is a zoonotic infection caused by *Toxoplasma gondii* (*T. gondii*), a protozoan with a worldwide distribution. Humans can become infected by the ingestion of raw or undercooked meat containing tissue cysts or the consumption of food or water contaminated with sporulated oocysts [1]. *T. gondii* parasite is an obligate intracellular opportunistic protozoan that can infect most species of warm-blooded animals including humans and has an unparalleled range of intermediate hosts, and is believed to infect up to a third of world population as well as a wide variety of animal species [2]. Moreover, toxoplasmosis is an opportunistic infection that can cause severe complications in immunocompromised persons [3,4]. The overall risk that acute *T. gondii* infection could result

in a congenital infection is estimated to be about 30%, with higher risks during the third trimester [5].

Cats are the definitive hosts of the parasite, as they place infectious stages in their feces, and it was found that these stages have very high rates of resistance to environmental conditions and thus play a major role in the spread of infection [6]. The transmission of toxoplasmosis to humans in many countries of the world is affected by several factors, the most important of which are people's cultures and customs, climate, and behavioral practices such as keeping cats [6], food preparation habits [7], and eating raw meat [8], drinking unclean water [9], and socioeconomic conditions play a role in the spread of infection [10], and it was noted that the seroprevalence of toxoplasmosis is associated with the age factor and the increase in the incidence of advanced age [11].

Pregnant women are infected with *T. gondii* during pregnancy, and the parasite is transmitted from the mother to the fetus through the placenta and associated with an increased risk of premature birth, miscarriage, stillbirth, and postnatal malformation [12,13]. The severity of symptoms and congenital infection depend on the stages of pregnancy and gestation age. Early diagnosis and health care for neonatal and maternal congenital toxoplasmosis are important in preventing consequences and complications [14]. Early diagnosis and treatment, particularly during the first trimester, have the potential to significantly improve overall clinical prognosis [15]. To date, there are no clinically effective vaccines to prevent this infection [16].

Toxoplasmosis is an important health problem in Yemen because of the region's geography, weather, health awareness, social habits, lifestyle, food and environment that help spread this parasite and increase its infection. Also, data on the seroprevalence of toxoplasmosis among pregnant women and girls in childbearing age in Mukalla city, Hadhramout are limited. Our findings can improve the management of *Toxoplasma* infections in this area. So, in this study, we aimed to understand the epidemiological status of *T. gondii* infection among Hadhrami pregnant women and identify the potential influencing factors.

Materials and Subjects

Study design and period

A cross-sectional and analytical study was carried out in the period from March to June 2022.

Sample size and study population

Non-probability (convenience sampling) was used, so that maximal participation ensured, the included 104 of pregnant women.

Pregnant women who underwent prenatal examination at the obstetrics and gynecology clinics in Mukalla city, Hadhramout were enrolled in the study.

Collection of blood samples

Blood samples were collected according to the steps of the InTec Products, INC company, China. Trained medical technicians collected 3 mL of elbow venous blood from each participant. Serum was separated from whole blood by centrifugation at 3000 rpm for 5 min. The separated serum was labeled and stored at -20°C until analysis.

Serological assay

Anti-*T. gondii* antibody tests were performed using the commercially available advanced quality one step TOXO-IgM and IgG immunochromatographic immunoassay for the detection of antibodies to *T. gondii* kits (InTec Products, INC company, China) according to the manufacturer's instructions. Positive and negative controls were included. Frozen samples were thawed, and cassette kit reagents were brought to room temperature (20–25 ◦C) before testing. All tests were performed manually, and all experiments were performed twice by the same researcher to ensure the repeatability of results.

Data collection tool

We collected information on potential risk factors, by means of a questionnaire that included a group of sociodemographic, clinical and behavioral variables.

Ethics approval and consent to participate

The study was approved by the ethical review board of Faculty of Science, Hadhramout university. All patients gave written informed consent, and participants data were anonymized.

Statistical analysis

Statistical analyses were performed using statistical package for social sciences (SPSS) (version 19.0 software). Data were presented as numbers and percentages. Differences in the seroprevalence of *T. gondii* were assessed using univariate analysis and bivariate analysis and were considered statistically significant at $P < 0.05$.

Results

General characteristics of the participants

It was clear from the questionnaire data that the age group of the targeted pregnant women 20-30 years represented the most in number 78(75.0%), and the secondary and then primary educational groups were the most numerous 47(45.2%) and 42(40.2%), respectively. Urban women were more numerous 80(77%) than rural women. The number of housewives was 91(87.5%). The

frequency of pregnancies 2-3 times and more than 3 times was 43(41.4%) and 36(34.6%) respectively, the largest number of previous one-time miscarriages/miscarriages was 25(24.0%), and a previous infection with toxoplasmosis was 17(16.3%), as shown in Table (1).

Table (1): General characteristics of the participants

Variable	Category	No. of Examined	*T. gondii* sero-Status (No.=104)	
			No.	%
Age groups (years)	13-20	11	5	4.8
	21-30	78	47	45.2
	31-40	15	7	6.7
Educational level	Illiterate	2	1	1.0
	Primary	42	20	19.2
	Secondary	47	21	20.2
	University	13	3	2.9
Residence	Urban	80	36	34.6
	Rural	24	9	8.7
Occupation	Student	4	1	1.0
	Housewife	91	41	39.4
	Job	9	3	2.9
Monthly income	Low	6	3	2.9
	Medium	89	40	38.5
	High	9	2	1.9
Family members/persons	3-5	50	18	17.3
	5-9	36	18	17.3
	More than 9	18	9	8.7
Pregnancy	Once	25	13	12.5
	2-3	43	25	24.0
	More than 3	36	21	20.2
Miscarriage	Non	73	40	38.5
	1	25	15	14.4
	2-3	5	4	3.8
	More than 3	1	0	0
Washing vegetables and fruits	Yes	88	39	37.5
	No	16	6	5.8
Illness history	Yes	17	9	8.7
	No	87	36	34.6
Eating restaurant foods	Yes	47	22	21.2
	No	57	23	22.1
Eating raw foods	Yes	15	11	10.6
	No	89	34	32.7
Contact with soil	Yes	49	21	20.2
	No	55	24	23.1
Contact with cats	Yes	14	10	9.6
	No	90	35	33.7
Contact with cat feces	Yes	17	11	10.6
	No	87	34	32.7
Water source	Filtered	68	26	25.0
	Non filtered	36	19	18.3

Seroprevalence of T. gondii in pregnant women

Table (2) shows the serological patterns of *T. gondii* in pregnant women. The serological prevalence of IgM antibody was 11(10.6%), which represents recent infections, while the serological prevalence of IgG antibody was 48(46.2%), which represents previous infections (protected) with the parasite, and 59(56.7%) were secondary infected (IgM and IgG positive).

Table (2): Serological patterns in pregnant women for *T. gondii* infection

Serological Patterns	No.	%	Status
Positive Toxo-IgM only	11	10.6	Recently infected
Positive Toxo-IgG only	48	46.2	Protected
Positive Toxo-IgG with positive Toxo-IgM	59	56.7	Secondary infected

Risk factors and their relationship to the prevalence of T. gondii infection in pregnant women

Univariate analysis of factors associated with *T. gondii* seroprevalence among the pregnant women in this study showed the possible transmission methods of the parasite, which eating raw foods (OR = 4.449, CI: 1.311-15.093, p = 0.017), being often in contact with cats (OR = 3.929, CI: 1.143-13.502, p = 0.030) and being often in contact with cat feces (OR = 2.858, CI: 0.967-8.449, p = 0.058) were significantly associated with seropositivity, as presented in Table (3)

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Table (3): Univariate analysis of factors associated with *T. gondii* seroprevalence among the pregnant women

Variable	No. of examined	Sero-positivity IgG		Sero-negativity IgG		COR	CI(95%)	P-value
		No.	%	No.	%			
Age groups (years)								
13-20	11	5	4.8	6	5.8	1	1	
21-30	78	47	45.2	31	29.8	0.45	0.154-1.958	0.356
31-40	15	7	6.7	8	7.7	0.048	0.200-4.538	0.951
Educational level								
Illiterate	1	1	1.0	1	1.0	1	1	
Primary	22	22	21.2	20	19.2	0.091	0.053-15.516	0.948
Secondary	26	26	25.0	21	20.2	0.192	0.048-13.698	0.882
University	13	10	9.6	3	2.9	0.7	0.014-6.382	0.440
Residence								
Urban	80	44	42.3	36	34.6	1.364	0.535-3.479	0.516
Rural	24	15	14.4	9	8.7	1	1	
Occupation								
Student	4	3	2.9	1	1.0	1	1	
Housewife	91	50	48.1	41	39.4	2.460	0.247-24.550	0.443
Job	9	6	5.8	3	2.9	1.500	0.106-21.312	0.765
Monthly income								
Low	6	3	2.9	3	2.9	1	1	
Medium	89	49	47.1	40	38.5	0.184	0.156-4.267	0.810
High	9	7	6.7	2	1.9	0.714	0.030-2.692	0.274
Family members/person								
3-5	50	32	30.8	18	17.3	0.437	0.189-1.672	0.301
5-9	36	18	17.3	18	17.3	1	0.323-3.101	1
More than 9	18	9	8.7	9	8.7	1	1	
Pregnancy								
Once	25	13	12.5	12	11.5	1	1	
2-3	43	25	24.0	18	17.3	0.22	0.289-2.102	0.623
More than 3	36	21	20.2	15	14.4	0.226	0.277-2.161	0.625

Miscarriage								
None	73	40	38.5	33	31.7	0.000	0.000	1
1	25	15	14.4	10	9.6	0.000	0.000	1
2-3	5	4	3.8	1	1.0	0.000	0.000	1
More than 3	1	0	0	1	1.0	1	1	
Washing vegetables and fruits								
Yes	88	49	47.1	39	37.5	1	1	
No	16	10	9.6	6	5.8	0.246	0.252-2.256	0.613
Illness history								
Yes	17	8	7.7	9	8.7	1.594	0.561-4.525	0.381
No	87	51	49.0	36	34.6	1	1	
Eating restaurant foods								
Yes	47	25	24.0	22	21.2	1.301	0.596-2.838	0.509
No	57	34	32.7	23	22.1	1	1	
Eating raw foods								
Yes	15	4	3.8	11	10.6	4.449	1.311-15.093	0.017*
No	89	55	52.9	34	32.7	1	1	
Contact with soil								
Yes	49	28	26.9	21	20.2	0.031	0.445-2.107	0.936
No	55	31	29.8	24	23.1	1	1	
Contact with cats								
Yes	14	4	3.8	10	9.6	3.929	1.143-13.502	0.030*
No	90	55	52.9	35	33.7	1	1	
Contact with cat feces								
Yes	17	6	5.8	11	10.6	2.858	0.967-8.449	0.058*
No	87	53	51.0	34	32.7	1	1	
Water source								
Filtered	68	42	40.4	26	25.0	0.446	0.245-1.254	0.156
Non filtered	36	17	16.3	19	18.3	1	1	

*Significant statistics at p-value <0.05
COR, Crude Odds Ratio; CI, Confidence interval

Multivariate analysis for significant variables associated with *T. gondii* infection among pregnant women confirmed that eating raw foods (Adjusted OR = 4.428, CI: 1.249-15.698, p = 0.021) was significantly associated with seropositivity of *T. gondii* infection, as shown in Table No. (4).

Table (4): Multivariate analysis of the predictors of *T. gondii* seroprevalence among pregnant women

Variables	Adjusted OR	95% CI	P-value
Eating raw foods	4.428	1.249-15.698	0.021*
Contact with cats	1.935	0.366-10.222	0.437
Contact with cat feces	2.158	0.495-9.421	0.306

*Significant statistics at p-value <0.05
OR, Odds Ratio; CI, Confidence interval

Discussion

In the present study, the total seroprevalence for anti-*Toxoplasma* antibodies, IgG and IgM was 56.7%. This seroprevalence found in the current study is higher than the seroprevalence rates of previous studies conducted in Yemen 45.4% and 46.2% respectively [17,18]. There are various explanations behind Yemen's high prevalence of toxoplasmosis such as living situations, economic and environmental factors, a lack of public health awareness, a lack of sanitary facilities and infrastructure, and a lack of access to safe drinking water.

The seroprevalence found in the present study is higher the seroprevalence rates of other studies conducted in different countries, Saudi Arabia 14.1% and 24.1% respectively [19,20], Ethiopia 38.8% [21], Peru 35.8% [22], Taiwan 7.7% [23], and China 3.6 % [12], but lower than the seroprevalence rates of previous studies

conducted in Brazil 68.6% [24], Brazilian Amazon municipality 68.3% [25], and Ethiopia, 70.8% [26]. However, this was close to the result (52.6%) obtained from Benin [27] and (40.9%) obtained from Saudi Arabia [19]. The variation in *T. gondii* prevalence across different countries can be attributed to factors that influence oocyst sporulation and survival in the environment [28]. Environmental, geographical location and characteristics of study subjects, such as age, educational level, cat handling, hygiene, and feeding habits play a crucial role in prevalence [29]. Also, infections tend to be more common in hot climates and low-lying areas compared to cold climates and mountainous regions, as well as in humid environments versus dry ones [20,30].

The seroprevalence rate of IgG and IgM antibodies in this study were 46.2% and 10.6% respectively. However, this was close to the result (IgG antibody was 44% and IgM antibody 11%) obtained from Sana'a [31], but higher than reported in other Yemeni governorates, Dhamar, (IgG antibody was 13.62% and IgM antibody 4.33%) [32], Hodeidah (14.5% for the IgG antibody and 14.4% for the IgM antibody) [33], and Ibb (for IgG antibody was 13% and for IgM antibody 4%) [34].

Our data showed a high prevalence of a possibility active toxoplasmosis in the study population. About 10.6% of pregnant women attending antenatal clinics had IgM, which indicates the possibility of early or acute infection of *T. gondii*, highlighting the high risk of placental transmission to the fetus and, consequently, congenital toxoplasmosis. The presence of IgM without IgG Ab may indicate an early infection, while the presence of both IgG and IgM Abs suggests acute infection [35]. However, this prevalence of IgM antibody is higher than reported in studies conducted in China 0.1% [12], Saudi Arabia 6.2% [20], Iran 2.27% [36], Brazil 0.25% [37], Ethiopia 9.3% [38], Algeria 0.89% [39], and Zambia 0.7% [40], but lower than the 12.93% and 12.5% reported in Erbil, Iraqi Kurdistan and Kuwait respectively [41,42].

The presence of IgG Ab, in our study about 46.2%, may indicate that infection with toxoplasmosis among these pregnant women had occurred in the past very commonly (chronic infection). Compared to other studies conducted in Yemen showed a seroprevalence of IgG Ab of 43.7, 27.9%, and 12.9% respectively [17,43,44]. High seroprevalence of IgG 58.5% was also reported from Brazil [37]. In Saudi Arabia, a neighboring country, the prevalence of IgG was 20% [20]. However, high prevalence of IgG reported in different studies conducted in Iran 38.63% [36], Ethiopia 76.4% [38], Turkey 26.3% [45], Syria 58.3% [46], and Burkina Faso 37.3% [47], but low prevalence showed in Kuwait 2.1% [42], Algeria 13.6% [39], and Zambia 4.2% [40]. However, it should be noted that comparing the seroprevalence of anti-*Toxoplasma* Abs between the different studies is problematic due to the different populations tested, their sociodemographic factors, and the use of different serological methods may also be responsible for discrepancies.

IgG antibodies become detectable 14 days after the first positive IgM test whereas IgM antibodies are detectable approximately 7 days after infection. IgG antibodies persist for approximately 2 years, whereas IgM antibodies tend to disappear within about 6 months after infection [12,48]. This difference in the development and persistence of IgG and IgM antibodies in serum may contribute to the higher seroprevalence of IgG in our study. Another possible explanation is that infected individuals seldom seek medical care promptly because the disease is usually asymptomatic, and the short persistence of IgM antibodies in the serum makes the detection of IgM antibodies more difficult [12].

The findings of this study suggest that toxoplasmosis, as assessed by risk factors, are not associated with educational level, age groups, occupation, residence, family members, monthly income, pregnancy period, washing vegetables and fruits, miscarriage, eating restaurant foods, illness history, contact with soil, and water source. This result means that these commonly hypothesized risk factors may not even significantly impact the transmission patterns of *T. gondii* in the target population. Other previous studies showed no association was found between seropositivity and the suspected risk factors assessed [40,49].

In this study, bivariate analysis showed contact with cat feces was significantly associated with anti-*T. gondii* antibodies, highlighting the importance of this route of transmission, as reported in previous studies [44,50]. This significant risk factor could be explained by the habit of keeping cats in the Yemeni community, which acts as a potential source for contamination of the soil, and the Yemeni climate, which sustains the infectivity of oocysts. The oocysts of *T. gondii* can remain infective for many months in suitable environment conditions such as warm temperature, moisture, and shade, which is the typical and relatively stable environment in Yemen [18]. The present study indicated that contact with cats is a significant risk factor for seropositivity. Previous studies conducted in Yemen [17,43], Ghana [51], Africa [52], and Burkina Faso [47] have reported a significant association between contact with cats and toxoplasmosis. However, these factors were not identified as independent risk factors of *T. gondii* infection in multivariate analysis in this study. Eating raw foods was significantly associated with the seroprevalence of *T. gondii* in the bivariate analysis. These findings are in agreement with recent studies [39,50,52]. Eating raw foods may play a significant role in disease transmission [1]. The multivariate logistic regression analysis showed

that eating raw foods was found to be statistically significant risk factor associated with toxoplasmosis seroprevalence among pregnant women. This finding is consistent with previous report in Cameroon [53].

Limitations

Despite obtaining valuable data concerning the seroprevalence of toxoplasmosis in Mukalla city within the scope of the present study, some limitations should be considered. The cross-sectional design of this study restricts causal conclusions about the identified risk factors. Additionally, the use of commercially available advanced quality immunochromatographic immunoassay for the detection of antibodies to *T. gondii* kits.

Conclusion

We concluded that there is a high seroprevalence of acute and chronic toxoplasmosis among pregnant women in Mukalla district; this is an alarming situation that necessitates public awareness, health education programs regarding the detrimental effects of toxoplasmosis in pregnant women. It is necessary prevention, early diagnosis at early gestation stage, and confirmation tests by avidity testing or PCR, in addition to the application of food and hygienic practices by the population.

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Author information

ORCID

Eidha Bin-Hameed: 0000-0002-0999-0173

مقالة بحثية

عدوى داء المقوسات بين النساء الحوامل في مدينة المكلا، حضرموت، اليمن: العوامل المرتبطة بالانتشار المصلي

عيظه بن حميد[1،2،*]، أمنية باوزير[1]، جواهر المهتدي[1]، رانيا باصريح[1]، سمية اليزيدي[1]، فاطمة بحاح[1]، ندى بايزيد[1]، و وردة بامرضاح[1]

[1] قسم علوم الحياة، كلية العلوم، جامعة حضرموت، اليمن
[2] قسم العلوم الصحية، كلية الطب والعلوم الصحية، جامعة العلوم والتكنولوجيا، عدن، اليمن

* الباحث الممثّل: عيظه بن حميد؛ البريد الالكتروني: e.binhamiad@hu.edu.ye و e.binhameed@ust.edu

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المُلخّص

داء المقوسات هو أحد أمراض الأوليات التي يسببها طفيل المقوسة القوندية، والذي يصيب مجموعة واسعة من العوائل، بما في ذلك البشر، حيث تُعتبر القطط العائل الرئيسي. قد يحدث انتقال خلقي للعدوى مما قد يؤدي إلى وفاة الجنين أو حديثي الولادة أو تشوهات خلقية شديدة. هدفت هذه الدراسة إلى تحديد معدل الانتشار المصلي وعوامل الخطورة المرتبطة بعدوى طفيل المقوسة القوندية بين النساء الحوامل في مدينة المكلا/حضرموت، اليمن. أجريت دراسة مقطعية وتحليلية من مارس إلى يونيو 2022 في مدينة المكلا، حضرموت. تم تسجيل النساء الحوامل اللواتي تتراوح أعمارهن بين 13 و40 عامًا في عيادات أمراض النساء والتوليد في الدراسة. تم جمع البيانات الاجتماعية والديموغرافية والعوامل المهيئة والبيانات السريرية لكل مشاركة من خلال استبيان. تم إجراء اختبار مصلي (فحص الكروماتوغرافيا المناعية) للكشف عن الأجسام المضادة وتحديد داء المقوسات الكامن بالكشف عن الأجسام المضادة نوع IgG، وداء المقوسات النشط بالكشف عن الأجسام المضادة نوع IgM. تم استخدام تحليل الانحدار اللوجستي لتحديد عوامل الخطورة المرتبطة بالإيجابية المصلية. من بين 104 مشاركة، كانت نتائج الأجسام المضادة نوع IgG المحددة لطفيل المقوسة القوندية إيجابية لدى 46.2%، بينما كانت نتائج الأجسام المضادة نوع IgM لدى 10.6% تشير إلى إصابة أولية حديثة. وقد أظهرت المشاركات اللاتي تتراوح أعمارهن بين 20 و30 عامًا أعلى معدل انتشار مصلي للأجسام المضادة IgG وIgM. أظهر تحليل الانحدار اللوجستي أحادي المتغير أن تناول الأطعمة النيئة (OR = 4.449, CI: 1.311-15.093, p = 0.017)، والتماس المباشر مع القطط (OR = 3.929, CI: 1.143-13.502, p = 0.030)، والتماس المباشر مع براز القطط (OR = 2.858, CI: 0.967-8.449, p = 0.058) ارتبطت ارتباطًا معنويًا بإيجابية المصل لطفيل المقوسة القوندية. وأكد التحليل المتعدد المتغيرات أن تناول الأطعمة النيئة (AOR = 4.428, CI: 1.249-15.698, p = 0.021) كان عامل تنبؤ مهم لانتشار طفيل المقوسة القوندية بين المشاركات في الدراسة. تشير نتائج الدراسة إلى أن طفيل المقوسة القوندية منتشرة على نطاق واسع في المنطقة، مما يؤدي إلى ارتفاع خطر تعرض النساء الحوامل بالإصابة. لتجنب العواقب الوخيمة المحتملة على الجنين، هناك حاجة ملحة لإجراء فحص منهجي خلال رعاية ما قبل الولادة وزيارات عيادات أمراض النساء وحملات التوعية الصحية.

الكلمات المفتاحية: داء المقوسات القوندية؛ الانتشار المصلي؛ النساء الحوامل؛ الحمل؛ عوامل الخطورة.

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