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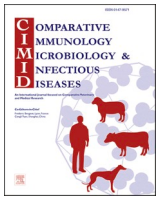

Full length article

Cross sectional survey on the prevalence and associated risk factors of toxoplasma infection in pregnant women in Biskra (Southeastern Algeria)

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ABSTRACT

Toxoplasmosis, caused by *Toxoplasma gondii*, is a zoonotic disease with significant global public health implications. In Algeria, data on its epidemiology are scarce. This study aimed to assess for the first time the seroprevalence of toxoplasmosis and identify associated risk factors among pregnant women in Biskra governorate in southeastern of Algeria. A cross-sectional survey (October 2022–May 2023) involved 453 women. Data on sociodemographics characteristics, gynecological history, and lifestyle habits were collected via structured questionnaires. Plasma samples were analysed for IgG and IgM antibodies using ELISA techniques. Statistical analyses, including univariate and multivariate logistic regression, were performed to identify significant risk factors associated with seropositivity. The overall seroprevalence of toxoplasmosis was 30.02 %. Univariate analysis identified several significant associated risk factors, including a history of spontaneous abortion (OR = 3.897), having single spontaneous abortions (OR = 4.96), consumption of unpasteurized milk (OR = 1.789), and owning pets (OR = 1.593). Living in urban areas appeared to be a protective factor (OR = 0.60). Multivariate analysis further highlighted feeding raw meat to animals (AOR = 8.395) and having given birth to a malformed child (AOR = 6.718) as major risk factors. Additionally, fast food consumption (AOR = 2.07) and cats ownership (AOR = 3.724) were also significantly associated with *T. gondii* seropositivity. The findings underscore the importance of implementig robust screening, prevention, and treatment strategies for toxoplasmosis, particularly among pregnant women. This study offers valuable epidemiological insights into toxoplasmosis in Algeria, addressing a critical knowledge gap.

1. Introduction

Toxoplasmosis is a parasitic infection caused by *Toxoplasma gondii*, which can be horizontally transmitted to humans through various routes, such as contact with contaminated soil, ingestion of undercooked meat containing cysts, or ingestion of oocysts in food or water [1]. Alternative transmission methods include organ transplantation [2], blood transfusion [3], congenital transmission and consumption of unpasteurized milk [1].

T.gondii infection is generally asymptomatic but may progress to chronic forms depending on the individual's immune status [4], particularly in pregnant women. If a woman acquires the infection for the first time during the early stages of pregnancy, the parasite might be transmitted to the fetus. This is known as vertical congenital transmission [5]. If congenital transmission takes place in the first period of conception, severe outcomes may results, including spontaneous abortions, neonatal death, hydrocephalus, microcephaly, intracranial calcifications, retinochoroiditis, strabismus, blindness, epilepsy, psychomotor and mental retardation, petechiae due to thrombocytopenia and anemia [6]. Therefore, the severity of infection depends on the gestational age at which the mother is exposed and the effectiveness of the placental barrier [7]. A global meta-analysis reported that

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annually, between 201 and 600 children are born with congenital toxoplasmosis [8].

T. gondii infection is typically diagnosed using various serological techniques, including the Indirect Fluorescent Antibody Test (IFAT), Enzyme-Linked Immunosorbent Assay (ELISA), Enzyme-Linked Fluorescence Assay (ELFA), Modified Agglutination Test (MAT), Western Blot (WB), Latex Agglutination Test (LAT) and Indirect Hemagglutination Test (IHA). These methods enable the distinction between acute and latent toxoplasmosis [9]. In addition, several molecular techniques are employed, such as polymerase chain reaction (PCR), nested PCR, real-time PCR, multiplex PCR, loop-mediated isothermal amplifcation (LAMP) and PCR–restriction fragment length polymorphism (PCR–RFLP) [10]. The development and diversification of diagnostic tests have contributed to a significant increase in recent publications investigating the epidemiology of *T. gondii* infection [11].

In Algeria, the government has commendably included congenital toxoplasmosis screening in its national surveillance program for pregnant women, providing this service free of charge. However, this beneficial initiative is unfortunately not available in the Biskra region. The high cost of these tests often leads many women to skip this crucial screening, even when their doctors recommend it, due to the financial constraints.

Approximately one-third of the global population were seropositive for latent toxoplasmosis, with significant variability across different countries and regions [12,13]. However, limited data are available on this parasitic infection in Algeria, particularly in the Biskra region, where no previous studies have been conducted. Therefore, it is crucial to assess the prevalence of the parasite and investigate associated risk factors, especially among pregnant women. This study aims to determine the seroprevalence of *T. gondii* and identify potential risk factors associated with *T.gondii* infection among pregnant women in Biskra governorate (Southeastern Algeria). The findings will provide the first comprehensive overview of the toxoplasmosis situation in this region and contribute to improved disease prevention and management strategies.

2. Material and methods

2.1. Area and duration of the study

This study was conducted in the governorate of Biskra, located in the south-eastern steppe of Algeria. It is situated approximately 440 Km from the capital, Algiers (Fig. 1) it spans an area of around 10,500 Km^2. Its altitude is 125 m above sea level, at a latitude of 34°5′ north and a longitude of 5°44′ east. Its climate is arid, with cold, dry winters and hot, dry summers [14]. The study was carried out from October 2022 to May 2023. The cross-sectional study focused on pregnant women who presented for antenatal screening of *T. gondii* infection antibodies. The participants typically attended medical laboratories following a recommendation from their doctors, who recommend *T. gondii* analysis, as part of routine screening for a new pregnancy or as a follow-up in cases where previous serological tests were negative.

2.2. The data origin

The required sample size was calculated based on the national estimate of toxoplasmosis seroprevalence reported by the Institut Pasteur d'Algérie (IPA), which is approximately 50 % among pregnant women. Using a margin of error of 5 %, the minimum sample size was determined to be 384 participants, calculated with the formula: $\mathbf{n = [z^2 \times p \times (1 - p)] / m^2}$; where **n:** is the sample size, **p:** is the estimated prevalence (50 %), **m:** is the margin of error (5 %), and **z** corresponds to a 95 % confidence level ($\mathbf{z} = 1.96$) [15]. A total of 453 of plasma samples from pregnant women were included in the analysis.

2.3. Inclusion and exclusion criteria

Blood samples from pregnant women were collected from multiple medical laboratories across the governorate of Biskra. The serological analysis for *T.gondii* antibodies was subsequently performed at the Laboratory of Genetic, Biotechnology and Valorization of Bioressources (LGBVB). Women were excluded from the study if they met any of the following criteria: attending for a subsequent visit, residing outside the governorate of Biskra, refusing to participate in the study, or having

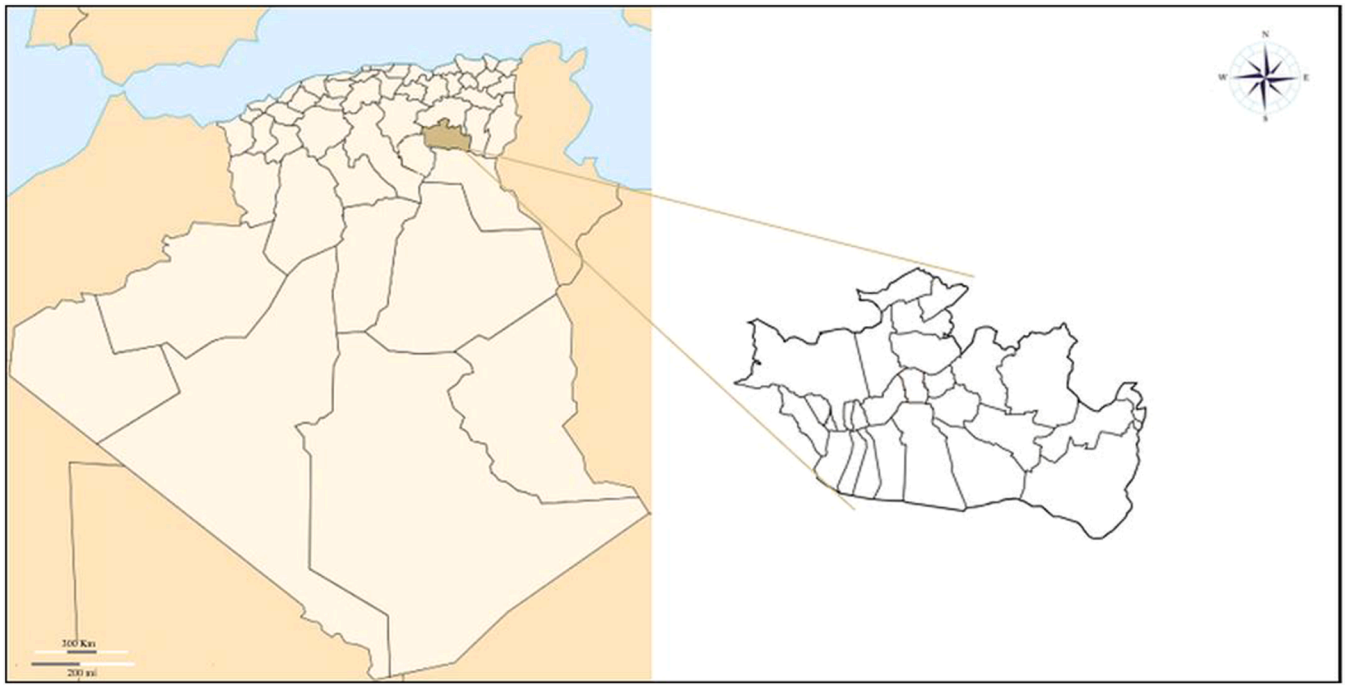

Fig. 1. Geographical location of Biskra region (Algeria).

incomplete data.

2.4. Survey and data collection

Following the verbal consent of participating women, a cross sectional survey was conducted by a medical assistant who interviewed the patients using a structured questionnaire. This questionnaire covered various items; including socio-demographic characteristics; (age, residence, profession, educational level, knowledge about toxoplasmosis), gynecological history; (gestational age, gravidity, history of diseases, spontaneous abortion, number of spontaneous abortions, malformed children, children with ocular anomalies) and lifestyle habits (source of water used, unpasteurized milk consumption, consumption of undercooked meat, fast food consumption, hand washing habits after soil contact, use of the same utensils for meat and vegetables, owning pet, cat ownership, cleaning of animal survival areas, raw meat consumption for animals). The questionnaire was pre-tested with a small group of 30 pregnant women to identify any misunderstandings, or data entry issues. This process allowed for improvements in the clarity and consistency of the questions.

Data confidentiality was strictly maintained, with responses securely recorded in paper forms. Operational definitions of spontaneous abortion, malformed children, and owning pet were clearly explained to the participants to ensure accuracy and consistency during analysis.

2.5. Sample collection and laboratory analysis

Once the questionnaire has been filled out, seven milliliters of venous blood were collected from each pregnant woman using disposable needles in vacutainer tubes containing the anticoagulant lithium heparin. The collected blood was centrifuged, before the exhaustion of 2 h after sampling, at 3000 G at room temperature for 15 min to recover the plasma. The plasma was divided into aliquots in microcentrifuge tubes, which was then transported in a cooler to the LGBVB laboratory and stored at −20 °C until analysis.

The serological diagnosis of toxoplasmosis was performed by determining both immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies from plasma samples.

The plasma samples were analyzed using a commercially available standard ELISA kit (Enzyme-Linked Immunosorbent Assay) marketed in Algeria and manufactured by EUROIMMUN, Lübeck, Germany, following the manufacturer's instructions. A Toxoplasma-specific IgG antibody titer $\geq$ 3 IU/mL was considered positive in this study.

The quantitative determination of specific IgM antibodies was also carried out using the EUROIMMUN ELISA system, which is widely used in Algerian laboratories. According to the manufacturer's interpretation guidelines, IgM concentrations with an index value $>$ 0.65 were considered positive, values $<$ 0.4 as non-reactive, and those between 0.4 and 0.5 as indeterminate.

2.6. Statistical analysis

SPSS Inc. "IBM SPSS Statistics 26" software, Chicago, IL, USA was utilized for statistical analysis, the Chi-square $\chi 2$ test was employed to assess the significance of the difference between *T.gondii* antibody seropositivity and all qualitative risk factors evaluated, with a statistically significant (*P*-value $<$ 0.05). Data were summarized as percentages, and univariate and multivariate analysis were generated using Binary Logistic Regression analyses to determine crude odds ratios (COR) and adjusted odds ratios (AOR) with 95 % confidence intervals ($IC_{95\ \%}$).

3. Results

Four hundred and fifty-three plasma samples from pregnant women were tested for the presence of anti-*T.gondii* IgG and IgM antibodies using the ELISA method. The total seroprevalence found in this study was 30.02 % (136/453). Two samples were positive for both IgG and IgM, one case showed IgM+ /IgG- profile, and 133 were IgM-/IgG+ , while the rest samples were negative for both IgM and IgG (Table 1).

The age of the participants was divided into four groups: $<$ 20, 20–29, 30–39 and $\geq$ 40 years. The highest estimated exposure to *T. gondii* was found in the group aged 30–39 years (33.2 %, 76/229), among those living in rural areas (37 %, 61/165), in participant with primary education (53.3 %, 8/15), and among participants who had no knowledge of toxoplasmosis, (31.6 %, 49/155). Furthermore, infection was slightly higher in employed women (30.4 %, 65/214) compared to unemployed individuals (29.7 %, 71/239) (Table 2). All sociodemographic characteristics showed a *P*-value $>$ 0.05, indicating no association with *T. gondii* antibody seropositivity, except for the "residence" variable, which was significantly associated with *T. gondii* antibody seropositivity ($P = 0.015$) (Table 2).

The analysis of gynaecological data showed the highest seroprevalence: in women in the third trimester (31.8 %, 28/88), in primigravida patients 30.4 % (31/102), and in patients with a history of disease, especially those who had undergone organ transplantation, 100 % (1/1). Among women with a history of spontaneous abortion, 61.1 % (11/18), in women with single spontaneous abortion, reaching 66.7 % (8/12) and in women who had malformed children showed a high positive prevalence of 71.4 % (5/7), also in women who have children with ocular anomalies (Table 2).

Women with a history of spontaneous abortion, number of abortion and who had malformed children showed a significant association with toxoplasma seropositivity (*P*-value of 0.003, 0.01, 0.016; respectively) (Table 2).

Analysis of lifestyle habits showed a high seroprevalence of toxoplasmosis antibodies among women who consumed tap water, with a percentage of 41.2 % (7/17), among women who did not consume undercooked meat; the seroprevalence was 30.02 % (130/433). Seroprevalence was also high among women who consume unpasteurized milk was 41.3 % (26/63), in women who usually eat fast food in restaurants 38.8 % (42/108). Additionally, the seroprevalence was 35.5 % (38/107) among women who wash their hands after gardening or soil contact, 37.7 % (46/123) among women who own pet, 61.5 % (32/52) in women who have contact with cats, 30.6 % (77/252) among women who do not use the same utensils for meat and vegetables,35.7 %(15/42) women who clean animals survival area and 60.0 % (39/65) among women who feed raw meat to animals (Table 2).

A significant association risk factor with *T.gondii* infection was found among women who consumed unpasteurized milk ($P = 0.036$), among women who own pet ($P = 0.037$), in women who have contact with cats (*P*-values $<$0.01) and in women who feed raw meat to their animals (*P*-values $<$0.01) (Table 2).

The univariate regression analyses identified five risk factors significantly associated with *T. gondii* infection. These included women having a history of spontaneous abortion (OR = 3.897), having single spontaneous abortions (OR = 4.96), consumption of unpasteurized milk (OR = 1.789), and owning pets (OR = 1.593). Living in urban areas appeared to be a protective factor (OR = 0.60) (Table 3).

Multivariate analysis further highlighted feeding raw meat to animals (AOR = 8.395) and having given birth to a malformed child (AOR = 6.718) as major risk factors. Additionally, fast food consumption (AOR = 2.07) and cats contact (AOR = 3.724) were also significantly associated with *T. gondii* seropositivity (Table 4).

Table 1
Distribution of *T. gondii* antibodies among women (N = 453) tested by ELISA.

ELISA results	IgM- / IgG-	IgM+ / IgG -	IgM- / IgG+	IgM+ / IgG+
Number of patients sampled (%)	317 (70.0 %)	1 (0.22 %)	133 (29.36 %)	2 (0.44 %)

Table 2
Risk factors related to IgG and IgM seroprevalence of *T. gondii* in pregnant women.

Characteristic	Number tested (%)	Positive serum n (%)	*P*-value
Age group			
<20 years	8 (1.8)	0 (0.0)	
20–29 years	203 (44.8)	56 (27.6)	
30–39 years	229 (50.6)	76 (33.2)	
≥ 40 years	13 (2.9)	3 (23.1)	0.126
Residence			
Urban	288 (63.6)	75 (26.0)	
Rural	165 (36.4)	61 (37.0)	0.015
Employment status			
Employed	214 (47.2)	65 (30.4)	
Unemployed	239 (52.8)	71 (29.7)	0.877
Education level			
Uneducated	4 (0.9)	1 (25.0)	
Elementary	15 (3.3)	8 (53.3)	
Intermediate	45 (9.9)	17 (37.8)	
Secondary	92 (20.3)	25 (27.2)	
University	297 (65.6)	85 (28.6)	0.211
Knowledge about toxoplasmosis			
Yes	298 (65.8)	87 (29.2)	
No	155 (34.2)	49 (31.6)	0.594
Gestational age			
First trimester	105 (23.2)	32 (30.5)	
Second trimester	260 (57.4)	76 (29.2)	
Third trimester	88 (19.4)	28 (31.8)	0.895
Gravidity			
Primigravida	102 (22.5)	31 (30.4)	
Multigravida	351 (77.5)	105 (29.9)	0.926
Historic diseases			
Good health	244 (53.9)	68. (27.9)	
Anemia	99 (21.9)	34 (34.3)	
Covid	50 (11.0)	15 (30.0)	
Cancer	10 (2.2)	2 (2.0)	
Organ transplant	1 (0.2)	1 (100)	
Other	49 (10.8)	16 (32.7)	0.495
Spontaneous abortion			
Yes	18 (4.0)	11 (61.1)	
No	435 (96.0)	125 (28.7)	0.003
Number of spontaneous abortions			
None	435 (96.0)	125 (28.7)	
One	12 (2.6)	8 (66.7)	
≥ Two	6 (1.3)	3 (50.0)	0.010
Malformed children			
Yes	7 (1.5)	5 (71.4)	
No	446 (98.5)	131 (29.3)	0.016
Children with ocular anomalies			
Yes	31 (6.8)	14 (45.2)	
No	422 (93.2)	122 (28.9)	0.057
Water source			
Tap	17 (3.8)	7 (41.2)	
Mineral	84 (18.5)	25 (29.8)	
Source	285 (62.9)	80 (28.1)	
Well	11 (2.4)	1 (9.0)	
Unknown	56 (12.4)	23 (41.1)	0.132
Unpasteurized milk			
Yes	63 (13.9)	26 (41.3)	
No	390 (86.1)	110 (28.2)	0.036
Undercooked meat			
Yes	20 (4.4)	6 (30.0)	
No	433 (95.6)	130 (30.0)	0.998
Fast food consumption			
Yes	108 (23.8)	42 (38.8)	
No	141 (31.1)	38 (27.0)	
Sometimes	204 (45.0)	56 (27.5)	0.070
Washing hands after soil contact			
Yes	107 (23.6)	38 (35.5)	
No	346 (76.4)	98 (28.3)	0.156
Use same utensils for meat/ vegetables			
Yes	201 (44.4)	59 (29.3)	
No	252 (55.6)	77 (30.6)	0.781
Owning a pet			
Yes	123 (27.2)	46 (37.3)	
No	330 (72.8)	90 (27.3)	0.037
Cat contact			
Yes	52 (11.4)	32 (61.5)	
No	401 (88.5)	104 (25.9)	< 0.01
Cleaning animals' area			
Yes	42 (9.3)	15 (35.7)	
No	411 (90.7)	121 (29.4)	0.398
Feeding raw meat to animals			
Yes	65 (14.3)	39 (60.0)	
No	388 (85.7)	97 (25.0)	< 0.01

Table 3
univariate logistic regression analysis for significant risk factors associated with T.gondii antibody seropositivity.

Variables	Crude odds ratio (95 % CI)	*P* value*
History of spontaneous abortion		
Yes	3.897(1.477–10.282)	**0.006***
No	Ref	
Number of spontaneous abortion		
No one	Ref	**0.01***
single abortion	4.960(1.4674–16.768)	
Two or more	2.480(0.494–12.453)	
Unpasteurized milk		
Yes	1.789(1.034–3.094)	**0.038***
No	Ref	
Residence		
Urban	0.60(0.398–0.906)	**0.015***
Rural	Ref	
Owning pet		
Yes	1.593(1.028–2.469)	**0.037***
No	Ref	

* statistical significance (P < 0.05. OR >1 and OR <1: association of variable with Toxoplasmosis. Ref: reference=1

Table 4
univariate and multivariate logistic regression analysis for significant risk factors associated with *T.gondii* antibodies seropositivity.

Variables	*T.gondii* antibodies seropositivity			
	Crude odds ratio (95 % CI)	*P - value**	Adjusted odds ratio (95 % CI)	*P-value**
Malformed children				
Yes	6.011 (1.152–31.378)	0.03*	6.718 (1.098–41.116)	0.039*
No	Ref		Ref	
Fast food				
Yes	1.725 (1.008–2.950)	0.047*	2.070 (1.103–3.882)	0.02*
sometimes	1.026 (0.633–1.662)		1.126 (0.637–1.991)	
No	Ref		Ref	
Feeding raw meat for animals				
Yes	4.5 (2.604–7.775)	< 0.01 *	8.395 (2.819–25.004)	< 0.01 *
No	Ref		Ref	
Cat contact				
Yes	4.569 (2.503–8.339)	< 0.01 *	3.724 (1.388–9.996)	0.009*
No	Ref		Ref	

* statistical significance (P < 0.05); OR > 1 and OR < 1: association of variable with Toxoplasmosis, Ref: reference= 1, ND: Not defined

4. Discussion

Few studies of *T.gondii* prevalence have been conducted in Algeria, and none have been carried out in the Biskra region. According to the literature, measurements of *T.gondii* seroprevalence vary considerably from one study to another. Therefore, additional data are needed to effectively plan and implement control and prevention strategies for the disease in this region.

The overall seroprevalence in this study was 30.02 % for total antibodies, indicating *T. gondii* infection. Compared to studies conducted in other Algerian regions, our results were different.

The seroprevalence of *T. gondii* observed in the present study is lower than that reported by Messerer (2014) [16]; (47.8 % in Annaba region), by Bachi et al. (2019) [17] (47.03 % in Algiers region among pregnant women) and slightly lower than the rates reported by Chouchane (2023) [18] (32.6 % in Setif region). Belkacem and Saidani (2015) [19] (34.5 % in the Tizi-Ouzou region), and Benramache et al. (2021) [20] (33.29 % in the Constantine region between 2016 and 2020). These studies consistently reported higher seroprevalence rates compared to the current findings. This variation may be largely explained by geographical and climatic differences. Northern Algeria (central, eastem and westen regions), characterized by a humid Mediterranean climate, provide more favorable environmental conditions for the survival and persistence of *T. gondii* oocysts in soil and water. Oocysts are known to remain viable for extended periods in moist environments, significantly increasing the likelihood of human exposure. In contrast, the much drier and arid climate of our study area may limit oocyst survival, thereby reducing the overall risk of infection. [21]

However, our findings were higher than the results of other studies reported by Khames et al. (2020) [22] in Medea region (25 %). Not far from the Biskra region climate, Mohamdi (2023) [23] found a seroprevalence of 28.3 % for *T.goondii* among pregnant women in Batna region. These differences may reflect regional variations in environmental exposure, hygiene practices, or dietary habits.

Compared to countries in the Maghreb region (North Africa), *T.gondii* seroprevalence in the present study was lower than that reported in Tunisia by Fakhfakh et al. (2013) [24] (47.7 %) and in Libya by elgodwi et al. (2020) in Tripoli region [25] (41.0 %) and in Morocco by Laboudi et al. (2021) [26] (43 %).

However, there is considerable variation between African countries in the prevalence of *T. gondii* in pregnant women, which may be due to differences in environment, climate and animal fauna. For Central and Eastern Africa, Mulu Gelaw et al. (2024) [27] estimated an overall seroprevalence of 37.1 % for *T. gondii* in women, a value very close to the results of our study. Nevertheless, Singh et al. (2021) [28] found a seroprevalence of 57.25 % in Ghana, and even higher, Adugna et al. (2021) [29] reported a seroprevalence of 70.82 % in Ethiopia, which was significantly and especially associated with the habit of consuming raw beef, reported by Negero et al. (2017) [30]. In Egypt, Abdelbaset et al. (2020) [31] reported a seroprevalence of 22.9 %, which is much lower than that found in our study, but with substantial variations between different Egyptian localities.

In northern Mediterranean countries, the prevalence of *T. gondii* varies considerably, with rates of 36.7 % in Turkey over the last 30 years [32], and 31.3 % in France between 1995 and 2016 [33]. Conversely, it does not exceed 25 % in Italy and spain [34,35]. This variation may be due not only to screening practices, such as in Italy, where testing is covered by the public health system [36], but also to environmental factors. Research has shown that the incidence of *T.gondii* infection is higher in humid and warmer climates [33].

Consequently, the differences in toxoplasmosis prevalence observed in different countries could be related to environmental factors, geographical conditions and levels of humidity [31]. Additionally, limited access to diagnostic services in our region may contribute to differences in screening rates and toxoplasmosis prevalence compared to other countries.

Epidemiological studies on the seroprevalence of *T.gondii* in pregnant women in different regions of Algeria showed a low seroprevalence estimated at 14.5 % in 2024 [21].The decrease in the prevalence of toxoplasmosis in recent years may be due to the hygienic habits adopted by women during the COVID-19 pandemic period. It's also been suggested that women now are more aware of the dangers of toxoplasmosis during pregnancy, following counselling and education programs provided in antenatal clinics [37].

Differences in prevalence may be due to the sample sizes studied and the heterogeneity of diagnostic methods, particularly serological immunoassays. Therefore, the validation of diagnostic tests is crucial for epidemiological studies, as the reliability of these tests directly influences the accuracy of prevalence estimates [38].

In the present study, the overall seroprevalence of *T. gondii* was not influenced by factors such as age, pregnancy duration, gravidity, water source, or consumption of undercooked meat. These findings are consistent with those reported by Sebaa et al. (2024) [21] in Djelfa governorate. In addition, there was no significant association with history of disease, cleaning of animal survival areas, washing hands after soil contact, or using the same utensils for vegetables and meat. According to Petersen et al. (2010) [39], up to two thirds of infections could not be explained by these risk factors.

However, other studies contradict our findings. For example, Khames et al. (2020) [22] found a significant association between increasing maternal age and the risk of toxoplasmosis in Medea region. Messerer et al. (2014) [16] also reported that the consumption of undercooked meat was a significant risk factor. The lack of a significant association between undercooked meat consumption and our study results may be due to effective sterilization methods. It is well established that tissue cysts can be eliminated by proper cooking, and commercial practices such as freezing and salting [40].

In this study, the highest seropositivity rates were observed among women aged 30–39 years, which may be attributed to their higher representation in the study population. Although advancing age is generally associated with increased exposure to *T. gondii*, the limited number of older participants may have influenced the absence of a significant trend in this group. Similar associations between age and toxoplasmosis seropositivity have been reported in other studies [22, 41].

In the present study, a significant association was observed between *T. gondii* seroprevalence and the place of residence of the pregnant women ($P = 0.015$). In particular, women living in urban areas were less likely to be positive for *T.gondii* infection (26.0 %; OR = 0.6) than those living in rural areas (36.4 %). This finding is consistent with the results reported by Nwachukwu et al. (2023) [42] in Nigeria, who also found a higher seroprevalence among women living in rural areas compared to their urban counterparts. The increased risk of infection in rural settings may be linked to lifestyle factors, including lower socioeconomic status, limited access to healthcare services, and increased exposure to *T. gondii* through frequent contact with domestic animals on farms [43]. However, other studies, have reported no significant difference in seroprevalence rates between rural and urban populations [22].

A statistically significant association was observed between *T. gondii* seropositivity and women with a historyof spontaeous abortion, particularly among those with a single abortion, who showed a higher odds ratio (OR = 4.96; $P = 0.01$). this finding suggests that *T. gondii* exposure during pregnancy could have contributed to fetal loss. A study conducted by Allamy et al. (2023) [44] confirmed the role of *T.gondii* parasite in causing abortion. According to Yoshifumi et al. (2008) [45], toxoplasmosis infection during pregnancy increases the levels of Th1 cytokines, especially IL-18 and IFN-γ, which induce excessive apoptosis of trophoblastic cells, possibly leading to abortion. These findings align with the results of Kalantari et al. (2021) [1], who established a significant association between history of spontaneous abortion and *T. gondii* seropositivity.

In the present study, having malformed child appears to be strongly

associated with *T.gondii* infection, with an adjusted odds ratio (AOR) of 6.7. this finding suggests that women who gave birth to a malformed child had a significantly higher risk of having being infected with *T. gondii* during pregancy, particularly, when primary maternal infection occurs in the first trimester [6]. Similar results were reported by Akubuilo et al. (2020) [45].

Many studies have reported the detection of *T. gondii* in milk from various of hosts, including cows [6], camels [46] and goats [47]. Dahmane et al. (2025) [47] confirmed that unpasteurised goat milk can serve as a source of human contamination with *T. gondii.* In addition, experimental studies have demonstrated that tachyzoites can survive in milk for three to seven days at + 4°C [48]. In Algeria, a high seroprevalence of *T. gondii* has been reported in goats (53.26 %) [48], followed by sheep (35.37 %) [49]. In our study, women who consumed unpasteurised milk showed a significantly higher association with *T. gondii* infection, with an odds ratio of 1.78 compared to those who do not consume unpasteurised milk **(**Table 3**)**. Similar results were reported by Rehman et al. (2020) [50].who identified raw milk consumption as a major risk factor for *T. gondii* infection.

Our study's multivariate logistic regression analysis revealed that regular fast food consumption was strongly associated with *T. gondii* infection, with an adjusted odds ratio (AOR) of 2.07, which was higher than that observed in women who rarely or never consumed fast food. This association may be explained by lifestyle factors, as many women, due to time constraints, frequently consume fast food, which may increase their risk of contracting toxoplasmosis. Foods such as sandwiches, burgers, patties and sausages may contain undercooked meat or grilled meat. In addition, unwashed green salads and raw vegetables pose an indirect risk due to potential contamination with *T. gondii* oocysts [51].

Cats are widely recognized as a significant risk factor for *T. gondii* transmission to intermediate hosts, including humans [51]. Consequently, cat ownership has been demonstrated to increase the risk of *T. gondii* infection in pregnant women [52]. Our investigation revealed significant associations between *T. gondii* infection and several animal-related factors, including pet ownership, cat contact, and feed raw meat to animals. Specifically, individuals who owned pets demonstrated a asignificant odds ratio of 1.593 for *T. gondii* seropositivity (Table 3), which aligns with results reported by Nguemaïm et al. [53]. Noting alsowomen who have a contact with cat showed a particularly elevated risk (adjusted odds ratio of 3.724) (Table 4). This finding aligns with similar studies conducted in the Annaba region [16].

These results are consistent with global patterns reported in a comprehensive meta-analysis covering 2000–2023, which confirmed that contact with cats is significantly associated with higher levels of anti-*T. gondii* antibodies [52]. However, conflicting evidence exists, as study conducted in Camerron by Nguemaïm et al. [53]; found no significant association between cats and toxoplasmosis infection.

Raw meat consumption is a well-established risk factor for *T. gondii* transmission due to the presence of tissue cysts containing infective bradyzoites. However, in the present study, no significant association was found between undercooked meat consumption and *T. gondii* infection in women, likely reflecting the low prevalence of this dietary habit in local culinary practices. Similar findings were reported by Sebaa et al. (2024) [21]. In contrast, a significant association was observed between feeding raw meat to animals and *T. gondii* seropositivity. Women who reported this practice exhibited a notably high adjusted odds ratio (AOR = 8.39), indicating a strong link between raw meat feeding and infection risk (Table 4). These results suggest that, beyond the direct transmission route through undercooked meat consumption, raw meat may also contribute indirectly to human infection by maintaining *T. gondii* reservoirs in domestic animals. This dual transmission pathway highlights the complex epidemiology of *T. gondii* infection in household settings.

5. Conclusion

In this study, the seroprevalence of *T. gondii* among pregnant women in the governorate of Biskra (Algeria) was estimated at 30.02 %, confirming the presence of the parasite in the local environment and its potential exposure to pregnant women. The risk of infection was significantly associated with rural residence, consumption of unpasteurised milk, a history of spontaneous abortion,particularly single abortions, having previously given birth to a malformed child, and frequent fast food consumption. In addition, owning pet, having contact with cats, and feeding raw meat to animals were identified as important risk factors. Based on these findings, routine antenatal screening and targeted health education programs focusing on toxoplasmosis transmission and risk prevention are strongly recommended for this region.

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CRediT authorship contribution statement

Djalel Eddine Gherissi: Writing – review & editing, Writing – original draft, Validation. **Adel Mammeri:** Validation, Resources, Formal analysis. **Nabil Mohamdi:** Validation, Investigation, Data curation. **TITAOUINE Mohammed:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Rayenne Benkacem:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Yahia Chebloune:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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