Seroepidemiology of *Toxoplasma gondii* Among Pregnant Women Attending Antenatal Care in Northwest Ethiopia

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Introduction: Toxoplasmosis is a life-threatening disease in pregnant women, having social and economic impacts due to congenital toxoplasmosis. However, in Ethiopia, it is neglected zoonotic disease, which requires screening and identifying risk factors in pregnant women to plan a public health intervention.

Methods: This institutional-based cross-sectional study was employed from March to April 2019 to estimate the seropositivity and assess potential risk factors for *T. gondii* among pregnant women attending antenatal care in four public hospitals in Northwest Ethiopia. Four hundred and one pregnant women were recruited to collect serum samples. *Toxoplasma gondii* antibodies were tested using Toxo-Latex slide agglutination test.

Results: Thus, the overall seropositivity for *T. gondii* was found 70.8% (95% CI: 66.3–75.5). Multivariable logistic regression analysis showed that the average monthly income, occupational status, habits of handwashing practices after handling raw meat, water sources for drinking and agroclimatic situations revealed significant ($p < 0.05$) effects on seropositivity for *T. gondii* in pregnant women.

Conclusion: This survey showed high seropositivity for *T. gondii* among pregnant women in Northwest Ethiopia. Therefore, further studies on bioassay, isolation and genotype of the pathogen are crucial. It also requires action to prevent and control the infection with *T. gondii* in pregnant women, which poses a potential threat to a fetus.

Keywords: Northwest Ethiopia, pregnant women, risk factors, seropositivity, *T. gondii*

Introduction

Toxoplasma gondii is important obligate intracellular coccidian that infects virtually all warm-blooded mammals and birds. It has been documented that the pathogen affects approximately 30% of the world human population.^{1–3} Food animals with infected tissue cysts are well-known sources of human infection. Humans and animals can acquire the pathogen via three main pathways: ingesting undercooked meat harbouring the tissue cysts of *T. gondii* or ingesting contaminated water and food with oocysts from the environment and congenital transmission from an affected mother to the fetus during pregnancy.^{1,4,5} Felids play a key role in the epidemiology of *T. gondii* because they are the only definitive hosts that excrete the viable oocysts in their faeces.^{1,3} Felids become infected with *T. gondii* by eating infected tissues from intermediate hosts.

The infection in immunocompetent individuals is asymptomatic and results in the chronic persistence of cysts within host tissues.^{1,3} But, in immunocompromised patients (such as cancer and HIV/AIDS patients); it causes life-threatening encephalitis

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due to the rupture of pre-existent cysts that reactivate latent infections.^{6,7} Moreover, the infection of pregnant women results in miscarriage or congenital infection that causes hydrocephalus, blindness, foetal death, neurological damage/intracranial calcification and retinochoroiditis.^{3,8,9} Recent reports have also reported that toxoplasmosis is a possible risk factor for personality shifts and increased likelihood of reduced intelligence or schizophrenia.¹⁰ Hence, early serological screening for toxoplasmosis in pregnant women is imperative.⁹

The prevalence of *T. gondii* infection in humans varies with the social and cultural habits of the community, geographical factors, cat lifestyle and density, wildlife structure, climate conditions and mode of transmission.⁴ Globally, the seroprevalence of *T. gondii* infection in humans ranges from 2% to 100%, depending on the source of the sample.⁴ In Ethiopia, seroprevalence estimates of 18.5–93.3% have been reported in different risk groups of the population.^{11–18} Similarly, in food animals, serological surveys indicate a high prevalence of *Toxoplasma* infections,^{19,20} and viable *T. gondii* isolates were also confirmed from tissues of food animals.²¹ Despite its high animal and public health significance, the wide practice of raw meat consumption, the keeping of cat as pet animals, presence of stray cats and suitable climatic conditions maintaining the survival of the pathogen, information on the seropositivity and associated risk factors of *Toxoplasma gondii* infection among pregnant women is still limited in the Northwest Ethiopia^{14,15,22} though there are several reports concentrated in the central and southern part of the country.^{11,16,18,23–25} Besides, though the parasite (*T. gondii*) is responsible for congenital infection resulting in abortion/still birth and neurological complications, no serological screening program during pregnancy in the country has been practiced. Therefore, this study aimed at estimating the seropositivity for *T. gondii* among pregnant women attending antenatal care and identifying possible risk factors associated with *T. gondii* infection in Northwest Ethiopia. The findings of this study would provide baseline information about the seropositivity rate and risk factors of *T. gondii* in pregnant women and also for planning and implementation of *T. gondii* antenatal screening, control and prevention strategies.

Materials and Methods

Study Areas

The study was conducted on pregnant women attending an antenatal care in the University of Gondar (located at

Gondar City), Kolladiba (Dembia district), Ambageorgis (Wogera district) and Addis-Zemen (Libokemkem district) hospitals located in Northwest Ethiopia. Gondar city is located 742 km far from Addis Ababa, capital city of Ethiopia. Its altitude is 2220 m above sea level (m.a.s.l). The rainfall pattern is bimodal, with an annual average of 1172 mm and mean annual temperature of 20°C. Wogera district has an average altitude of 2900 m.a.s.l in the northern highlands of Ethiopia. The rainfall pattern is bimodal, with an annual average of 700 mm and mean annual temperature of 12.7°C. Dembia district has an elevation ranges from 1500 to 2500 m.a.s.l and the area is mainly midland. Libokemkem has an average elevation of 2000 m.a.s.l. The rainfall pattern is unimodal, with an annual average of 1300 mm and a mean annual temperature of 19.7°C.

Study Design and Population

This institutional-based cross-sectional study was employed from March to April 2019 in Northwest Ethiopia to estimate seropositivity and assess the risk factors for *T. gondii* in pregnant women who attended the outpatient obstetric clinic. Four hundred and one pregnant women were recruited at the University of Gondar, Kolladiba, Ambageorgis and Addis-Zemen hospitals located in the Amhara regional state, Northwest Ethiopia. Pregnant women who were seriously ill and not consent to participate were excluded from this study.

Blood Sample Collection and Serological Analysis

Blood samples were collected by trained medical laboratory personnel to have serum for serological analysis for *T. gondii*. Approximately 5 mL of the venous blood sample was drawn from each consenting study participant aseptically using a plain tube. Test tubes were labelled properly with the necessary information. Then, the whole blood was left for an hour at room temperature to allow clotting and then centrifuged at 3000 rpm for 10 min to separate serum. The serum was collected using 1.5-mL Eppendorf serum tubes and then, transported using an icebox to the Parasitology Laboratory, College of Veterinary Medicine and Animal Sciences, University of Gondar. The serum samples were kept at –20°C until the serological test was made using a commercial Toxo-Latex agglutination test (SPINREACT, S.A/S.A.U Ctra Santa Coloma, Spain) following the manufacturer's instructions.

Questionnaire Survey

A structured and pre-tested questionnaire was also used to gather information on sociodemographic characteristics, environmental, obstetrical/clinical and behavioural factors anticipated to be associated with *T. gondii* seropositivity in the study participants to get relevant information. The exit interview with the participants was made to gather the required data. The questionnaire was administered by trained health professionals.

Data Management and Analysis

The collected data using a questionnaire and laboratory analysis were stored in EPI Info version 7 and analysed using SPSS version 20.0. Descriptive statistics were used to summarize the result concerning the risk factors. The seropositivity rate was calculated as the number of seropositive serum samples divided by the total number of serum samples tested. Logistic regression was employed to identify the risk factors associated with *T. gondii* seropositivity. The independent variables with a *p*-value of ≤ 0.20 during the univariable logistic analysis were fitted into a multivariable logistic regression model to avoid the effect of confounders. Adjusted Odds Ratio (AOR) with the corresponding 95% confidence interval (CI) was estimated to show the strength of the association. A *P*-value < 0.05 was used to declare statistically significant in the final model. The model fitness was checked using the Hosmer–Lemeshow goodness-of-fit test.

Ethical Considerations

The study was performed in accordance with good clinical practices following the principle of the declaration of Helsinki.²⁶ Ethical clearance was received from the University of Gondar Ethics Committee (Ref. No: V/P/RCS/05/1237/2018). All volunteer study participants were informed about the study and written agreements were obtained from all involved women. Confidentiality was assured using codes.

Results

Characteristics of Study Participants

A total of 401 pregnant women aged between 18 and 46 years with a mean age of 26.8 ± 5.08 years attending ANC clinics at Addis-Zemen, Ambageorgis, Dembia, and University of Gondar hospitals were recruited and involved in this study. Nearly half of the study participants (53.1%) were found within the age group of 25–30 years

while 17% of them had more than 30 years of age. The majority (55.6%) of them were urban residents and 38.9% of them were unable to read and write. According to gestational age, 84 (20.9%), 169 (42.1%) and 148 (36.9%) were at the first, second and third trimester, respectively. Approximately 32.2% of the participants were housewives followed by governmental employees (24.7%), farmers (22.9%) and self-employed (traders) (20.2%). Half of the study participants had an average monthly income of 3001–6000 Ethiopian birr. Forty-seven percent of the study participants owned a cat in their home. The seropositivity for *T. gondii* and other demographic characteristics of the study participants is indicated in Table 1.

Toxoplasma gondii Seropositivity and Its Associated Risk Factors

In this survey, the overall seropositivity for *T. gondii* antibodies among pregnant women was found 70.8% (95% CI: 66.3% - 75.5%). *T. gondii* seropositivity was found to be higher among pregnant women from Dembia primary hospital (78%) followed by Addis-Zemen primary hospital (75%), Gondar comprehensive specialized hospital (68%) and Ambageorgis primary hospital (54.2%) as indicated in Figure 1.

Of the study pregnant women; 29.9% were between age groups of 18–24 years, 53.1% were between 25 and 30 years and 17% were over 30 years. The seropositivity of *T. gondii* among age groups 18–24, 25–30 and over 30 years was found to be 70%, 72%, 69%, respectively. However, no significant association was observed between seropositivity and age. There was no significant association between seropositivity for *T. gondii* and educational level and residence of the participant (Table 2).

None of the study participants had a history of organ transplantation; whereas 7% had a history of blood transfusion in their lifetime. Of those who had blood transfusion history, 86% were seropositive. From the participants, 19.2% had an abortion history of whom 75% were seropositive. From the study of pregnant women, 47.1% reported having a domestic cat at home. Of them, the seropositivity was found to be 69% (Table 3).

Assessment of raw meat eating habits revealed only a minority (23.2%) experienced eating raw meat; 76.8% had no experience of raw meat eating habits throughout their lifetime. Among those who had a raw meat-eating habit, the seropositivity for *T. gondii* was 73%. Similarly,

Table 1 Demographic Characteristics of Pregnant Women Attending ANC at Selected Hospitals, Northwest Ethiopia

Variables	Categories	Seroprevalence		Total n (%)
		Negative n (%)	Positive n (%)	
Age (Years)	18–24	36 (30)	84 (70)	120 (29.9)
	25–30	60 (28)	153 (72)	213 (53.1)
	>30	21 (31)	47 (69)	68 (17)
Residential	Rural	56 (31)	122 (69)	178 (44.4)
	Urban	61 (27)	162 (73)	223 (55.6)
Educational level	Illiterate	48 (31)	108 (69)	156 (38.9)
	Primary	16 (25)	49 (75)	65 (16.2)
	Secondary	20 (26)	58 (74)	78 (19.5)
	Above secondary	33 (32)	69 (68)	102 (25.4)
Occupational status	Governmental employee	36 (36)	63 (64)	99 (24.7)
	Traders (self-employed)	16 (20)	65 (80)	81 (20.2)
	Farmers	30 (33)	62 (67)	92 (22.9)
	Housewives	35 (27)	94 (73)	129 (32.2)
Gestational period	1st trimester	23 (27)	61 (73)	84 (20.9)
	2nd trimester	52 (31)	117 (69)	169 (42.1)
	3rd trimester	42 (28)	106 (72)	148 (36.9)
Average monthly income (ETB)	<1000	35 (31.5)	76 (68.5)	111 (27.7)
	1000–3000	53 (26)	149 (74)	202 (50.4)
	3001–6000	19 (28)	49 (72)	68 (17)
	≥6001	10 (50)	10 (50)	20 (5)

37.2% experienced eating uncooked vegetables. The seropositivity for *Toxoplasma* among those who experienced raw vegetable eating habit was 75% (Table 3).

Risk factor analysis was conducted using univariate and multivariate logistic regression. The results of univariate logistic regression analysis demonstrated that

occupational status, average monthly income (in ETB), history of blood transfusion, hand washing practice after handling raw meat, the habit of raw vegetable consumption, residence agroecology (Figure 2) and sources of drinking water had a p -value <0.2. These variables were considered for further analysis using multivariate logistic

Figure 1 Seropositivity for *T. gondii* in pregnant women among health facilities.

Table 2 Demographic Risk Factors Associated with *T. gondii* Seropositivity in Pregnant Women

Variables	Category	Total n (%)	Seropositive n (%)	COR (95% CI)	AOR (95% CI)
Age (years)	18–24	120 (29.9)	84 (70)	1.00	
	25–30	213 (53.1)	153 (72)	1.09 (0.67–1.79)	–
	>30	68 (17)	47 (69)	0.96 (0.50–1.83)	–
Residence	Rural	178 (44.4)	122 (69)	1.00	
	Urban	223 (55.6)	162 (73)	1.22 (0.79–1.88)	–
Educational status	Illiterate	156 (38.9)	108 (69)	1.08 (0.63–1.84)	–
	Primary	65 (16.2)	49 (75)	1.47 (0.73–2.95)	–
	Secondary	78 (19.5)	58 (74)	1.39 (0.72–2.67)	–
	Above secondary	102 (25.4)	69 (68)	1.00	
Occupation	Farmers	94 (23.4)	59 (63)	1.00	1.00
	Traders	102 (25.4)	82 (80)	2.43 (1.28–4.63)*	2.69 (1.28–5.63)**
	Governmental employee	92 (22.9)	62 (67)	1.23 (0.67–2.24)	1.33 (0.61–2.91)
	Housewife	113 (28.2)	81 (72)	1.50 (0.84–2.70)*	1.49 (0.80–2.80)
AMI (ETB)	<1000	111 (27.7)	76 (69)	2.17 (0.83–5.69)*	3.57 (1.19–10.64)**
	1000–3000	202 (50.4)	149 (74)	2.81 (1.11–7.13)*	3.81 (1.37–10.61)**
	3001–6000	68 (17)	49 (72)	2.58 (0.93–7.18)*	3.88 (1.27–11.84)**
	≥6001	20 (5)	10 (50)	1.00	1.00

Notes: * p -value <0.2, ** p -value <0.05.

Abbreviation: AMI, average monthly income.

regression analysis. Thus, occupational status, average monthly income of household (ETB), the residence agroecology, the habit of handwashing practices after handling raw meat and sources of drinking water were significantly associated with seropositivity for *T. gondii* (p -value <0.05). Accordingly, self-employed study participants (traders) (AOR = 2.53, 95% CI: 1.17–5.50, p = 0.019); participants having an average monthly income of <1000 (AOR = 3.57, 95% CI: 1.19–10.64, p = 0.023), 1000–3000 (AOR = 3.81, 95% CI: 1.37–10.61, p = 0.010) and 3001–6000 (ETB) (AOR = 3.88, 95% CI: 1.27–11.84, p = 0.017); irregular handwashing practices after handling raw meat (AOR = 1.93, 95% CI: 1.06–3.53, p = 0.031); midland climatic condition (AOR = 2.16, 95% CI: 1.23–3.63, p = 0.007) and unprotected well/spring water sources used for drinking (AOR = 2.37, 95% CI: 1.03–5.43, p = 0.042) were found to be the main predictors associated with *T. gondii* seropositivity among ANC attended pregnant women in selected hospitals located in Northwest Ethiopia (Table 3).

Discussion

Estimating of *T. gondii* seropositivity in pregnant women has a paramount significance because of the risk of infection of the foetus or new-born and reactivation of infection

in immunocompromised individuals. This study revealed overall seropositivity of 70.8% for *T. gondii* among ANC attending pregnant women in Northwest Ethiopia. Although the prevalence in this study is lower than the previous studies in Ethiopia,^{12,16,23,24} it has been noted that different serological tests and cut-off values were used so it is difficult to compare the studies. Alternatively, this study reported higher seropositivity than studies conducted elsewhere in the world.^{25,27–29} The variation in seropositivity might be due to variation in the geographical distribution of the parasite, climatic condition (dry or moist climate, temperature and relative humidity), cat population densities and feeding habits, the cultural pattern of the population (feeding habits) and socioeconomic status.^{3,7} It may also be varied due to the prevalence of the parasite in animals and the type of animal consumed. The difference is further attributed to the sampled population, genetic background of the parasite and the host, a type of immune response elicited by the parasite. The serological test method used and sensitivity difference could explain such discrepancies.^{15,18,24,27}

The current study showed a strong association between the average monthly income of pregnant women and seropositivity for *T. gondii*. This finding is in agreement with the previous study conducted in Zambia.³⁰ In contrast,

Table 3 Clinical, Behavioural and Environmental Risk Factors Associated with *T. gondii* Seropositivity in Pregnant Women

Variables	Category	Total n (%)	Seropositive n (%)	COR (95% CI)	AOR (95% CI)
Gestational period	1st trimester	84 (20.9)	61 (73)	1.18 (0.66–2.11)	–
	2nd trimester	169 (42.1)	117 (69)	1.00	–
	3rd trimester	148 (36.9)	106 (72)	1.12 (0.69–1.82)	–
History of abortion	No	324 (80.8)	226 (70)	1.00	–
	Yes	77 (19.2)	58 (75)	1.32 (0.75–2.34)	–
Child with visual impairment	No	394 (98.3)	278 (71)	1.00	–
	Yes	7 (1.7)	6 (86)	2.50 (0.3–21.03)	–
History of blood transfusion	No	373 (93)	260 (70)	1.00	1.00
	Yes	28 (7)	24 (86)	2.61 (0.88–7.69)*	2.87 (0.88–9.30)
Habit of raw meat consumption	No	308 (76.8)	216 (70)	1.00	–
	Yes	93 (23.2)	68 (73)	1.16 (0.69–1.95)	–
Handwashing practices after handling raw meat	Always	288 (71.8)	198 (69)	1.00	1.00
	Sometimes	102 (25.4)	78 (76)	1.48 (0.88–2.49)*	1.93 (1.06–3.53)**
	Never	11 (2.7)	8 (73)	1.21 (0.31–4.68)	1.22 (0.29–5.16)
Habit of raw vegetable consumption	No	252 (62.8)	172 (68)	1.00	–
	Yes	149 (37.2)	112 (75)	1.41 (0.89–2.22)*	1.41 (0.84–2.37)
Residence agroclimatic situations	Highland	75 (18.7)	44 (59)	1.00	1.00
	Midland	285 (71.1)	213 (75)	2.08 (1.23–3.55)*	2.16 (1.23, 3.63)**
	Lowland	41 (10.2)	27 (66)	1.36 (0.62–3.00)	1.34 (0.59, 3.03)
Sources of water	Hand pump	203 (50.6)	137 (67)	1.00	1.00
	Municipal pump	106 (26.4)	80 (75)	1.48 (0.87–2.52)*	1.16 (0.54–2.48)
	Unprotected well water	62 (15.5)	46 (74)	1.39 (0.73–2.63)	2.37 (1.03–5.43)**
	Protected well water	30 (7.5)	21 (70)	1.12 (0.49–2.59)	1.91 (0.66–5.51)
Cat presence at home	No	212 (52.9)	154 (73)	1.00	–
	Yes	189 (47.1)	130 (69)	0.83 (0.54–1.28)	–

Notes: *p-value <0.2, **p-value <0.05.

a study conducted in central Ethiopia showed the absence of a significant association between average monthly income and seropositivity for *T. gondii*.¹² More seropositivity was found among pregnant women with low socioeconomic status than high socioeconomic. This significant association could be due to pregnant women of low socioeconomic status being more prone to live and work in highly dense areas with poor sanitary conditions and lack good education on good hygienic practice.^{29,30}

In this study, a significant interaction was also observed between *T. gondii* seropositivity and occupational status of the study participants. Self-employed (traders) pregnant women were 2.53 times more likely to be seropositive for *T. gondii* compared to governmental employee participants ($p = 0.019$). This finding is in agreement with the study conducted in Nigeria.³¹ In contrast,

studies conducted in Tanzania³² and Ethiopia^{16,18} reported an insignificant association between occupational status and *T. gondii* seropositivity. The observed difference might be due to variation in the occupational classification of the study participant in this study. Additionally, self-employed (traders) pregnant women were more at risk to *T. gondii* seropositivity, probably due to more exposure to contaminated meat, vegetables and fruits, while working as merchants or seller (while working in the cafeteria or restaurants). Moreover, these workers might prefer outing, thus exposed to contaminated feed (undercooked meat, fruit and salad) and water.²⁹

The habit of handwashing practice after handling raw meat also showed a significant effect on the seropositivity of pregnant women for *T. gondii*. The odds of being seropositive for *T. gondii* was about two times more likely

Figure 2 Seroprevalence of *T. gondii* infection in relation with agroecology of residence.

to occur in those who have washed their hands irregularly after handling raw meat than those who always wash their hands after handling raw meat (AOR = 1.93, $p = 0.031$). It is in agreement with the previous study done in Sri Lanka.²⁸ In contrast, some studies report the absence of association between *T. gondii* seropositivity and hand-washing practice while handling raw meat.^{18,23} This significant association indicates that contaminated or infected raw meat could serve as a source of *T. gondii* infection during handling raw meat for preparation and selling or any purpose due to a high possibility of accidental ingestion of tissue cysts or tachyzoites from animal blood.²⁹

A statistically significant association was also documented between the source of drinking water and seropositivity for *T. gondii*. The odds of being seropositive for *T. gondii* were 2.37 times more likely to be observed in participants who used the unprotected well/spring water source for drinking purposes than those study participants who used the municipal pump water source for drinking ($p < 0.05$). This is in agreement with the previous study conducted.³³ Contrarily, the absence of a significant association between *T. gondii* seropositivity and water sources used for drinking were reported in other studies.^{14,15} The observed association between *T. gondii* seropositivity and water source in this study indicates that high contamination of wells/spring by *T. gondii* oocysts from cats' faeces and inadequate water management, which could serve as the source of *T. gondii* infection.¹⁸

Residence agroclimatic situations also showed a significant effect on seropositivity for *T. gondii* in

pregnant women in this study. Hence, those study participants who resided in the midland area were about two times more likely to be seropositive than those pregnant women resided in the highland areas (AOR = 2.16, $p = 0.007$). This conforms to the previous study conducted in Ethiopia.¹² This might be attributed to the difference in the climatic conditions, where higher seropositivity influenced by humidified, moist and wet areas, which are favourable for oocyst sporulation. Oocyst survival also increases and remains viable more than a year in a moist environment that results in high contraction of infection of human and animals.³⁴

Cats excrete millions of viable oocysts with their faeces in a short period and play a major role in the transmission of *T. gondii*. However, the current study showed that the presence of cats at home was not significantly associated with seropositivity for *T. gondii* although the prevalence was high in pregnant women. Similar findings were observed in various countries.^{16,18,28,35} However, this finding is inconsistent with several studies that reported as the owning cat significantly correlated with *T. gondii* seropositivity.^{11,14,15,36} The difference in the types of cats (stray and pet) and infection rates in cats may account for the observed differences.²⁴ Alternatively, study participants might acquire infection via different routes such as raw meat and vegetable consumption, contaminated water and from the contaminated environment (soil).

The insignificant interaction was observed between the gestational period and *Toxoplasma* seropositivity in this study. Corresponding findings were also reported in several studies.^{16,18,23,24} This study also demonstrated that the

habit of eating raw meat was not significantly associated with *T. gondii* seropositivity. Similar findings were also reported in other studies.^{16,23,37} In contrast, a significant association between *T. gondii* infection and eating raw/undercooked meat has been reported.^{35,38} This difference might be due to the frequency of raw meat consumed, the type of animal's meat consumed (cattle, sheep, goat, pig) and the prevalence of the parasite in the animals.²³ Moreover, this study showed that educational level, history of abortion, history of blood transfusion, the number of gravidities and habits of raw vegetable consumption had an insignificant effect on *T. gondii* seropositivity among pregnant women. This agrees with the report from southern Ethiopia.²⁵

In conclusion, the result of this finding revealed high *T. gondii* seropositivity among pregnant women in the study area. Of the predictors assessed in this study, having an average monthly income, occupational status, altitude, the habit of handwashing practices after handling raw meat and water sources for drinking were found to be important predictors that increase the odds of *T. gondii* seropositivity in pregnant women. Therefore, further studies on bioassay, isolation and genotyping of the pathogen are crucial. It also requires action to avert and control the infection with *T. gondii* in pregnant women, which poses a potential threat to the foetus.

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Disclosure

The authors report no conflict of interest in this work.

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