

ORIGINAL ARTICLE

**SEROPREVALENCE AND RISK FACTORS FOR HUMAN
TOXOPLASMOSIS IN NORTHEASTERN BRAZIL**

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ABSTRACT

Toxoplasmosis is one of the most prevalent parasitic infections in humans, causing severe clinical complications in immunocompromised patients or in foetuses. Estimates on the toxoplasmosis seroprevalence in human populations vary widely, even within the same region. This study aimed to estimate the prevalence of *Toxoplasma gondii* infection in the municipality of Santa Cruz, State of Rio Grande do Norte, Brazil, and identify the risk factors for toxoplasmosis. *T. gondii* IgG antibodies were measured in 66.2% of the studied population. The factors associated with infection were as follows: age above 45 years (OR = 7.4; 95% CI = 3.7-14.8); illiteracy (OR = 2.8; 95% CI = 1.6-5.0); the presence of more than three cats in the home (OR 2.0; 95% CI = 1.2-3.5); no water tank (OR = 2.0; 95% CI = 1.4-3.1); and consumption of raw or unpasteurized milk (OR = 2.0; 95% CI = 1.3-3.0). The risk factors identified were predominantly related to the ingestion of oocysts. This study may contribute to public health knowledge of toxoplasmosis and the definition of control programs, particularly for pregnant women from regions similar to the area studied.

KEY WORDS: *Toxoplasma gondii*; toxoplasmosis; seroepidemiology; transmission; Rio Grande do Norte.

INTRODUCTION

Toxoplasma gondii is a protozoan that is prevalent in most areas of the world, presenting significant medical and veterinary importance, as it causes miscarriage or congenital disease in its intermediate hosts (Tenter et al., 2000). Two types of transmission occur according to the different infective forms of

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this parasite: (a) horizontally, through the ingestion of oocysts present in the environment or the ingestion of tissue cysts found in raw or undercooked meat; and (b) vertically, by transplacental transmission of tachyzoites (Dubey, 1994). Given the asymptomatic nature of the majority of infections, it is technically difficult to determine their source(s). Geographical region, climate, and hygiene and nutritional habits cause the prevalence rate to vary significantly around the world (Flegr et al., 2014). Analysing the presence of antibodies to *T. gondii* in the Brazilian population, the prevalence was found to range from 36% in Minas Gerais (Maia et al., 2012) to 87.4% in Paraiba (Junior & Monteiro, 2010). There are multiple sources of *T. gondii* infection, and the variation observed in the prevalence of toxoplasmosis according to region reinforces the importance of identifying regional risk factors. Such studies are scarce in northeastern Brazil (Dubey et al., 2012). The purpose of this study was to estimate the seroprevalence of toxoplasmosis and its factors associated with infection among volunteers in the municipality of Santa Cruz, Rio Grande do Norte, in northeastern Brazil.

MATERIAL AND METHODS

Study Design and Area

A cross-sectional population-based study was carried out in the municipality of Santa Cruz, located in the State of Rio Grande do Norte, Brazil. Santa Cruz has a population of 35,797 inhabitants (Brasil, 2010), and is divided into five areas: Centro, Paraiso, Maracuja, DNER, and Conego Monte.

Sample Calculation and Sampling Process

To establish the minimum sample number, the OpenEpi programme, 3.03a version, was used. For sample calculation, the following data were considered: an average prevalence of toxoplasmosis in Brazil of 60% (Dubey et al., 2012), a population of 35,797 inhabitants, a confidence interval of 95%, and an estimated precision of 5%. Based on the information on the number of households per area, the sampling process was conducted proportionately, with the percentage of homes or families calculated for each street in each area. All the streets in the town were included, and the number of houses selected (randomly) was proportional to the size of the street block. The sample consisted of 1,540 individuals and all the selected residents were included in the study, as long as they complied with the inclusion criteria stating that all volunteers were to be Santa Cruz residents aged 13 or older.

This study was approved by the Ethics Committee of the Federal University of Rio Grande do Norte (CAAE 08922712.0.0000.5537). All the volunteers signed an Informed Consent Form.

Data collection

The interviews were conducted using a questionnaire including individual variable data such as demographic, socioeconomic and behavioural aspects, as well as household characteristics. Questions on eyesight problems, miscarriage, and disease awareness were also included in the questionnaire.

Blood sample collection and serological analysis

Blood was collected from each volunteer by puncturing a peripherical vein. An in-house ELISA (Buery et al., 2014) was used to test all serum samples. Samples were tested in duplicate. To assess the reproducibility (Szklo & Nieto, 2012) 10% of the sera samples were randomly selected and processed in duplicate in a masked test.

Statistical analysis

The database was generated using the Epidata, version 3.1, by double entry of the results and analyzed using STATA version 10.0. The prevalence of *T. gondii* infection was estimated according to overall population, gender, age group and area. To investigate the factors associated with *T. gondii* infection a mixed logistic regression model was used and the household was included as a random effect. The categorical variables were transformed into dummy variables. Univariate analysis was conducted for all variables collected, and those that attained a p value < 0.25 were included in the multivariate models. Backward analyses were used to construct a final model and variables with significance levels of p<0.05 were maintained.

RESULTS

1,540 volunteers from 1,217 families were included in the study. Of these, 256 families and 325 volunteers resided in the Centro area, 439 families and 581 volunteers resided in the Paraiso area, 216 families and 251 volunteers resided in Conego Monte area, 150 families and 187 volunteers resided in the Maracuja area, and 156 families and 196 volunteers resided in the DNER area. Individual and socio-economic characteristics in the municipality of Santa Cruz are presented in Table 1.

Table 1. Individual and socio-economic characteristics in the municipality of Santa Cruz, RN associated to seroprevalence of toxoplasmosis, 2013-2014.

Characteristics of the volunteers	N = 1,540	%
Gender		
Female	1,156	75.1
Male	384	24.9
Area		
Centro	325	21.2
Paraiso	581	37.7
Conego	251	16.3
Maracuja	187	12.1
DNER	196	12.7
Age (years old)		
≥ 13 to < 15	60	3.8
≥ 15 to < 30	365	23.7
≥ 30 to < 45	449	29.2
≥ 45 to < 60	348	22.6
≥ 60	318	20.7
Education level		
Illiterate	405	26.3
Up to the 4 th grade	340	22.1
Up to the 8 th grade	320	20.8
Secondary level	387	25.1
Higher education	88	5.7
Family incomes (Brazilian minimum wage) *		
< 2	963	79.6
≥ 2 to < 7	232	19.2
≥ 7	14	1.2
Knowledge about toxoplasmosis		
Yes	419	27.2
No	1,121	72.8
Complains about eyesight		
No	466	30.3
Yes	1,074	69.7
Has had miscarriage		
No	289	18.8
Yes	867	56.3

*The family income of 8 residences was not reported.

Seroprevalence of Toxoplasma gondii infection and univariate analysis

Analysis using ELISA-IgG showed that 1,020 (66.2%) out of the 1,540 volunteers tested positive for toxoplasmosis. Approximately 10% of the samples (153 sera) were tested in a masked trial, showing 100% agreement, with the kappa index equal to 1, indicating excellent reproducibility.

The prevalence of toxoplasmosis ranged from 59.2% in the DNER area to 70.2% in the Paraiso area (Table 2). Volunteer age analysis showed that higher age led to an increased prevalence of toxoplasmosis (Table 2, Figure 1A). Conversely, the higher the education level, the lower the prevalence of toxoplasmosis (Table 2).

Regarding gender, the prevalence of anti-*T. gondii* IgG antibodies showed that 64.9% of the women and 70.3% of the men tested positive for toxoplasmosis. The seronegative women (35.1%) diagnosed in this study were mostly (71%) of reproductive age, between 15 and 45 years (Figure 1B). Notably, 74.7% of the women with positive serology for toxoplasmosis reported a miscarriage. Considering variables related to the individual characteristics of the volunteers, univariate analysis showed males presenting a higher probability of infection (Table 2). Additionally, ages above 30 years presented higher proportions of positive individuals for toxoplasmosis. The comparative analysis of the areas showed a greater number of positive volunteers residing in the Paraiso and Centro areas. Illiterate volunteers had the highest probability of being seropositive. Volunteers presenting knowledge about toxoplasmosis had a lower probability of infection than those who had no information about the disease (Table 2). For people who reported eyesight problems, a significant difference was noted between the volunteers with and without toxoplasmosis (Table 2); those with toxoplasmosis reported more ocular problems than seronegative people.

Regarding diet and behavioural characteristics of the volunteers, there was a significant difference between volunteers who reported drinking water from wells, pipe trucks, rivers, and dams and those who did not drink from these types of water sources (Table 3). The same trend was observed regarding the consumption of raw or unpasteurized cow milk. Although volunteers did report eating undercooked pork, chicken, goat, sheep and, in particular, beef, no relationship to toxoplasmosis infection was identified through univariate analysis (Table 3).

Regarding housing characteristics, the analysis showed a higher probability of infection among individuals who had previously resided in houses with mud, dirt, or sand flooring than among those living in houses with cement or ceramic floors. Two other housing characteristics ($p < 0.25$) that resulted in a higher probability of infection were the absence of a water tank and the presence of more than 3 cats in the house (Table 4).

Table 2. Socio-economic, demographic, and behavioral characteristics of the volunteers in the municipality of Santa Cruz, RN, associated with seroprevalence of toxoplasmosis, 2013-2014.

Variables	ELISA Positive (%)	Negative (%)	Univariate OR (95% CI)	p*
Gender				
Female	750 (64.9)	406 (35.1)	1	
Male	270 (70.3)	114 (29.7)	1.3 (0.9 – 1.6)	0.05
Area				
DNER	116 (59.2)	80 (40.8)	1	
Maracuja	115 (61.5)	72 (38.5)	1.1 (0.7 – 1.7)	0.64
Conego	161 (64.1)	90 (35.9)	1.2 (0.8 – 1.8)	0.28
Centro	220 (67.7)	105 (32.3)	1.4 (1.0 – 2.1)	0.05
Paraiso	408 (70.2)	173 (29.8)	1.6 (1.2 – 2.3)	0.00
Age (years old)				
≥ 13 to < 15	25 (41.7)	35 (58.3)	1	
≥ 15 to < 30	173 (47.4)	192 (52.6)	1.3 (0.7 – 2.2)	0.41
≥ 30 to < 45	280 (62.4)	169 (37.6)	2.3 (1.3 – 4.0)	0.00
≥ 45 to < 60	264 (75.9)	84 (24.1)	4.4 (2.4 – 7.7)	0.00
≥ 60	278 (87.4)	40 (12.6)	9.7 (5.3 – 17.9)	0.00
Education level				
Illiterate	343 (84.7)	62 (15.3)	1	
Up to the 4 th grade	240 (70.6)	100 (29.4)	0.4 (0.3 – 0.6)	0.00
Up to the 8 th grade	190 (59.4)	130 (40.6)	0.3 (0.2 – 0.4)	0.00
Secondary level	202 (52.2)	185 (47.8)	0.2 (0.1 – 0.3)	0.00
Higher education	45 (51.1)	43 (48.9)	0.2 (0.1 – 0.3)	0.00
Knowledge about toxoplasmosis				
Yes	259 (61.8)	160 (38.2)	1	
No	761 (67.9)	360 (32.1)	1.3 (1.0 – 1.6)	0.03
Complains about eyesight				
No	686 (63.9)	388 (36.1)	1	
Yes	334 (71.7)	132 (28.3)	1.4 (1.1 – 1.8)	0.00
Has had miscarriage				
No	534 (61.6)	333(38.4)	1	
Yes	216 (74.7)	73 (25.3)	1.8 (1.4 – 2.5)	0.00

*Variables were considered significant at p<0.05.

Table 3. Diet and Behavioral Characteristics of the volunteers in the municipality of Santa Cruz – RN, associated with seroprevalence of toxoplasmosis, 2013 – 2014.

Variables	ELISA		Univariate OR (95% CI)	p*
	Positive (%)	Negative (%)		
Drinking water				
Mineral	167 (58.0)	121 (42.0)		1
Tap	672 (67.7)	320 (32.3)	1.5 (1.2 – 2.0)	0.00
Untreated	181 (69.6)	79 (30.4)	1.7 (1.2 – 2.4)	0.00
Drinks/drank well water				
No	174 (54.0)	149 (46.0)		1
Yes	820 (69.7)	357 (30.3)	2.0 (1.5 – 2.5)	0.00
Drinks /drank river water				
No	488 (60.6)	318 (39.4)		1
Yes	507 (72.9)	188 (27.1)	1.7 (1.4 – 2.2)	0.00
Drinks/drank pipe truck water				
No	280 (58.9)	195 (41.1)		1
Yes	717 (69.7)	312 (30.3)	1.6 (1.3 – 2.0)	0.00
Drinks / drank dam water				
No	336 (56.3)	261 (43.7)		1
Yes	659 (72.7)	247 (27.3)	2.0 (1.7 – 2.6)	0.00
Habit of drinking raw/ un pasteurized milk				
No	869 (64.7)	473 (35.3)		1
Yes	150 (76.1)	47 (23.9)	1.7 (1.2 – 2.4)	0.00
Habit of eating raw or undercooked beef				
No	790 (65,8)	411 (34,2)		1
Yes	230 (67,8)	109 (32,2)	1,9 (0,8 – 1,4)	0,48
Habit of eating raw or undercooked pork				
No	938 (66,1)	482 (33,9)		1
Yes	82 (68,3)	38 (31,7)	1,1 (0,7 – 1,6)	0,61
Habit of eating raw or undercooked goat meat				
Yes	957 (66,1)	490 (33,9)		1
No	63 (68,1)	30 (31,9)	1,0 (0,7 – 1,7)	0,75
Habit of eating raw or undercooked chicken				
No	919 (65,8)	477 (34,2)		1
Yes	101 (70,1)	43 (29,9)	1,2 (0,8 – 1,7)	0,30

*Variables were considered significant at $p < 0.05$.

Table 4. Presence of cats and housing characteristics of the volunteers in the municipality of Santa Cruz, RN, associated with seroprevalence of toxoplasmosis, 2013-2014.

Variable	ELISA		Univariate OR (95% CI)	P*
	Positive (%)	Negative (%)		
Housing characteristics (N=1,217)				
Flooring of the previous house **				
Cement or ceramic	644 (66.9)	318 (33.1)	1	
Mud / dirt / sand	110 (79.7)	28 (20.3)	1.9 (1.3 – 3.0)	0.00
Backyard floor				
Cement or ceramic	374 (68.2)	174 (31.8)	1	
Dirt	252 (64.4)	139 (35.6)	0.84 (0.6 – 1.1)	0.22
Mixed	88 (66.7)	44 (33.3)	0.93 (0.6 – 1.4)	0.73
Presence of water tank				
Yes	696 (65.5)	367 (34.5)	1	
No	119 (77.3)	35 (22.7)	1.2 (1.1 -1.4)	0.00
Have (or had) cat				
No	367 (61.78)	227 (38.22)	1	
Yes	448 (71.91)	175 (28.09)	1.6 (1.3 – 2.0)	0.00
How many cats have				
None	164 (68.9)	74 (31.1)	1	
1 – 2	214 (71.8)	84 (28.2)	1.1 (0.8 – 1.7)	0.46
> 3	70 (80.5)	17 (19.5)	1.9 (1 – 3.4)	0.04

*Variables were considered significant at $p < 0.05$.

**For 1,100 who reported having lived in another residence.

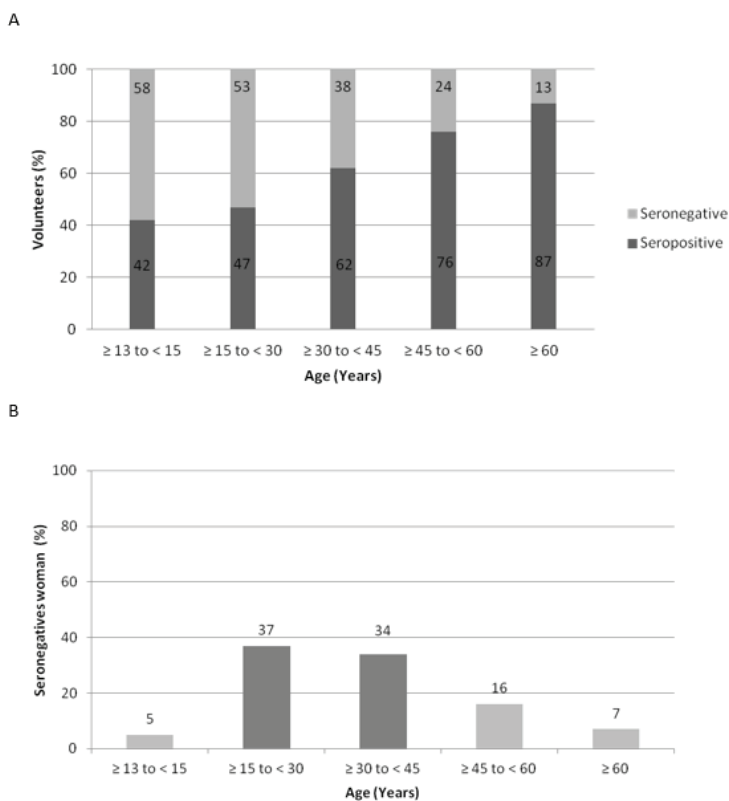


Figure. Relation between volunteer age and serology in the municipality of Santa Cruz, RN 2013-2014. A. Proportion, by age range, of volunteers seropositive and seronegative of toxoplasmosis. B. Distribution, by age range, only for seronegative women.

Multivariate analysis

The final model presented the following factors associated with infection by *T. gondii*: age, level of education, the presence of three or more cats in the house, not having a water tank, and consumption of raw or unpasteurized milk (Table 5). For age, the prevalence of toxoplasmosis was found to increase throughout life, indicating that individuals aged over 45 are 7.4 times more likely to be infected by the parasite than those aged between 13 and 15 years. Illiterate individuals were observed to be 2.8 times more likely to acquire toxoplasmosis than those with or without higher education.

The probability of exposure to the parasite increased according to the number of cats present in the house. In households with more than three cats, the inhabitants were twice as likely to be infected as in households without cats. The exposure to toxoplasmosis also increases two-fold when the individuals drank raw or unpasteurized milk or had no water tank at home (Table 5).

Table 5. Multivariate logistic analysis of risk factors for infection with *Toxoplasma gondii* in the municipality of Santa Cruz, RN, 2013-2014.

Variable	Univariate OR (95% CI)	Multivariate OR (95% CI)	p*
Age (years old)			
≥ 13 to < 15			
≥ 15 to < 30	1.3 (0.7 – 2.2)	1.8 (1.0 – 3.4)	0.06
≥ 30 to < 45	2.3 (1.3 – 4.0)	3.4 (1.8 – 6.6)	0.00
≥ 45	6.1 (3.5 – 10.6)	7.4 (3.7 – 14.8)	0.00
Education level			
Illiterate	5.3 (3.2 – 8.7)	2.8 (1.6 – 5.0)	0.00
Up to the 4 th grade	2.3 (1.4 – 3.7)	1.6 (0.9 – 2.8)	0.07
Up to the 8 th grade	1.4 (0.9 – 2.2)	1.6 (0.9 – 2.8)	0.07
Secondary level	1.0 (0.6 – 1.7)	0.9 (0.6 – 1.6)	0.96
Higher education			
How many cats have			
None			
1 – 2	1.1 (0.8 – 1.7)	1.5 (1.1 – 2.1)	0.05
≥ 3	1.9 (1 – 3.4)	2.0 (1.2 – 3.5)	0.01
Presence of water tank			
Yes			
No	1.2 (1.1 -1.4)	2.0 (1.4 – 3.1)	0.01
Habit of drinking raw/ un pasteurized milk			
No			
Yes	1.7 (1.2 – 2,4)	2.0 (1.3 – 3.0)	0.01

*Variables were considered significant at p<0.05.

DISCUSSION

In this study, a high prevalence of 66.2% was found for anti-*T. gondii* IgG antibodies in residents of the municipality of Santa Cruz, RN. Surveys carried out in other northeastern areas also showed a high seroprevalence of 66.4% in São Luis, MA (Costa et al., 2010), 87.4% in Joao Pessoa, PB (Junior & Monteiro., 2010), and 59% in Jaçana, RN (Freitas et al., 2017). Interestingly, seroprevalence did not vary considerably according to family income, as noted in other studies (Dias et al., 2011; Lopes-Mori et al., 2013; Benítez et al. 2017). This is possibly due to inaccurate reporting by the volunteers, who sometimes tended to avoid this question. The seroprevalence of toxoplasmosis increased progressively with age, confirming other studies conducted in Brazil (Ferreira et al., 2009; Lopes-Mori et al., 2013). Increased prevalence of *T. gondii* infection with age may be justified by the longer exposure to risk factors, such as environmental pollutants, nutrition, regional development, public water supply infrastructure, and health (Remington et al., 1995; Carellos et al., 2014; Benitez et al, 2017).

The main social impact of this protozoan infection is associated with vertical transmission. In this study, 71% of the seronegative women were of reproductive age between 15 and 45. Similarly, the study conducted in Minas Gerais State showed that young mothers, including teenagers, are significantly more affected by the disease (Carellos et al., 2014). Out of the 1,156 female volunteers in this study, 74.7% reported miscarriages and were also seropositive for toxoplasmosis; however, the causes of abortion were not investigated.

Five risk factors associated with infection were found, three of which were related to the individual characteristics of the volunteers (age, level of education and the consumption of raw or unpasteurized milk) and two regarding household characteristics (number of cats and no water tank available in the home). As previously mentioned, age is related to the duration of exposure to risk factors, and seroprevalence is the amount of accumulated exposure during a person's lifetime in a particular social setting (Flegr et al., 2014). The level of education variable can reflect the volunteer's degree of knowledge on the disease. The association of level of education with toxoplasmosis indicates the need to invest in education in the municipality of Santa Cruz, as 84.7% of those characterized as illiterate were seropositive for the disease. Additionally, a high percentage (67.9%) of the volunteers who had no knowledge of the disease showed positive serology for toxoplasmosis. A study in Natal, the capital of the State of Rio Grande do Norte, showed a lower prevalence of toxoplasmosis (21.5%) in students whose mothers had higher education than in those students (53.2%) whose mothers were illiterate or had not completed secondary education (Garcia et al., 2004). The association between level of education and toxoplasmosis was also observed in Pernambuco (Porto et al., 2008), Tocantins (Silva et al., 2014), and Paraná (Dias et al., 2011; Lopes-Mori

et al., 2013), where prevalence was higher in mothers with less than eight years of schooling than in those with eight years or more of schooling. Our results suggest health education can actually prevent infection by *T. gondii* because it aims to alter human behaviour, which is particularly relevant to pregnant women (Foulon et al., 2000; Moura et al., 2016).

Cats in the house led to an increased risk of infection by *T. gondii*, especially when three or more cats were present. Our data corroborate a study in the USA that showed that an increased risk of infection associated with exposure to cats was found to occur only in those who kept three or more animals (Jones et al., 2009). The larger number of cats may be associated with a higher number of oocysts released in household areas, which may reflect an increased risk of infection. Appropriate hygiene measures, such as proper disposal of cat faeces, frequent cat feeding and litter box cleaning using industrialized products, as well as the habit of eating well-cooked meat, can reduce the risk of infection (Jones et al., 2001).

The lack of a water tank in the home was significantly associated with toxoplasmosis in the multivariate model. The residents in the municipality of Santa Cruz constantly suffer from lack of water, being forced to save and store the water that reaches their homes. Those who do not have a water tank often store roof runoff rainwater in semi-open tanks. However, these tanks are subject to environmental contamination. Volunteers who kept cats (42%) reported that their animals defecated outside the home and even on the roofs. The oocysts in the faeces on the roof can leach due to rain and be deposited in these tanks, becoming a source of infection if the water is consumed without being appropriately treated. In Paraná, the consumption of water not originating from the public supply system resulted in an increased risk of infection by *T. gondii* (Dias et al., 2011).

The consumption of raw or unpasteurized milk does not seem to be a common habit in the municipality of Santa Cruz because only 12.8% of the volunteers reported having this habit. However, 76.1% of the volunteers who usually drink raw milk showed positive serology for toxoplasmosis; thus, this dietary habit is a risk factor for infection with *T. gondii*. It is likely that in these cases, infection occurs by ingesting tachyzoites found in the milk of animals with acute toxoplasmosis or oocysts due to external contamination of milk caused by lack of hygiene during milking. In São Paulo, the consumption of unpasteurized cow milk was observed in 19.4% of the study population, and exposure to this factor showed significant differences between infected and non-infected women (Galisteu et al., 2007). Similar results were also found in Tocantins (Silva et al., 2014) and Rio de Janeiro (Moura et al., 2013).

Our study shows the importance of toxoplasmosis in public health, indicating that it is essential that each country or region have its own epidemiological information to define control programmes, particularly for

pregnant women since both prevalence and incidence of toxoplasmosis vary from region to region within the same country.

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