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**MAJOR ARTICLES**

# Epidemiological factors associated with seropositivity for toxoplasmosis in pregnant women from Gurupi, State of Tocantins, Brazil

**Marcos Gontijo da Silva**[1]

**Joseneide Teixeira Câmara**[2]

**Marina Clare Vinaud**[3]

**Ana Maria de Castro**[3]

[1]Departamento de Parasitologia, Universidade Regional de Gurupi, Gurupi, TO
[2]Departamento de Enfermagem, Universidade Estadual do Maranhão, Caxias, MA
[3]Departamento de Parasitologia, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO



## ABSTRACT

### Introduction

Knowledge of the prevalence and risk factors for *Toxoplasma gondii* dissemination among pregnant women is relevant because the parasite can be spread from mother to infant. The objective of this study was to assess the epidemiology and risk factors of toxoplasmosis in pregnant women from Gurupi, State of Tocantins, Brazil, from February 2012 to June 2013.

### Methods

The study population included 487 pregnant women. Sociodemographic, dietary and cultural data were collected using a standardized and validated form. Peripheral blood was collected for serologic testing using the ELISA test (IgM/IgG antibodies). The data were analyzed by comparing seropositivity with risk factors using crude and adjusted odds ratios.

### Results

The prevalence rate for IgG and IgM antibodies was 68.7% and 5.7%, respectively. Sociodemographic characteristics associated with toxoplasmosis risk included the following: education level ≤ 8 years (OR: 6.612; CI: 1.450-30.144), age ≥ 30 years (OR: 5.273; CI: 1.166-23.844), working outside the home (OR: 1.604; CI: 1.015-2.536), and family income of two minimum wages or lower (OR: 2.700; CI: 1.891-8.182). Regarding dietary habits, there was a significant association of seropositivity with meat intake (OR: 1.78; CI: 1.149-4.080), cutting vegetables without

washing the cutting board beforehand (OR: 2.051; CI: 1.165-3.614), frequent intake of vegetables (OR: 2.051; CI: 1.368-3.006) and *in natura* milk intake (OR: 2.422; CI: 1.014-5.785).

**Conclusions**

The high prevalence rates of toxoplasmosis in Gurupi are related to age, raw meat and *in natura* milk intake, as well as education level, working outside the home, and poor hygienic habits during meal preparation.

**Key words:** Toxoplasmosis in pregnancy; Pregnant women; Risk factors; Gurupi/Tocantins

## INTRODUCTION

Toxoplasmosis during pregnancy is among the leading causes of neonatal morbidity and mortality[1]. The knowledge of risk factors present in a given population is important for the establishment of preventive actions and programs that may reduce the risk of adverse events during the fetal and neonatal periods[2,3].

*Toxoplasma gondii* is the etiologic agent of toxoplasmosis; after infecting pregnant women, it can reach the placenta through the blood and may infect the fetus, causing congenital toxoplasmosis[4]. New maternal infection during pregnancy can cause death of the embryo/fetus, symptomatic neonatal disease or late sequelae in the survivors[5,6].

Human toxoplasmosis may be considered a food-borne disease. Even if the infection is not directly related to food, one of the main routes of infection is the oral intake of oocysts or bradyzoite cysts. Other important routes of infection are the congenital route, blood transfusions, organ transplantations, and laboratory accidents, listed in order of decreasing frequency[7].

Determining which exposure factors to analyze should be based on knowledge of the biological cycle of the causative agent, i.e., the possible ways to ingest the oocysts in the environment or the bradyzoite cysts present in meat[8,9].

Therefore, proper knowledge of the risk factors associated with the prevalence of *Toxoplasma* infection is imperative to determine prophylactic measures. This study aims to identify risk factors for toxoplasmosis in pregnant women treated at public hospitals in Gurupi, State of Tocantins.

## METHODS

This is a cross-sectional analysis of the risk factors for toxoplasmosis in pregnant women from the City of Gurupi, Tocantins, from February 2012 to June 2013.

Eligibility and participation criteria stipulated that the patients must be pregnant women who agreed to take part in the research and provided informed consent.

The parameters used to estimate the sample size were a prevalence of 70%, a significance level (type I error) of 5% and a precision of 0.6%. A loss of 5% was also estimated.

A random sample of pregnant women treated in the Basic Health Unit (BHU) in the urban area of Gurupi was selected. The majority of pregnant women who sought prenatal care at the BHU were from the urban area of the city. After prenatal consultation, the women were taken to a private room to maintain privacy while they received information regarding the research and signed the informed consent form. They also answered a standardized and validated questionnaire.

After the interview, we collected 5ml of peripheral blood from each woman to perform immunoglobulin G (IgG) and immunoglobulin (IgM) enzyme-linked immunosorbent assays (ELISA). If the patient did not agree to take part in the study, they were excluded from the research.

To preserve internal validity, the same serological test (with the same kit and manufacturer) was used for all patients. Additionally, the analyses were performed at the same laboratory and by the same technician to avoid bias. The tests were processed at the Laboratory for the Study of Host-Parasite Relationship at the Tropical Pathology and Public Health Institute, Federal University of Goiás (LAERPH-IPTSP/UFG), which is a reference laboratory located in Goiânia, State of Goiás. Samples were frozen and transported in sealed Styrofoam coolers to maintain the characteristics of the sera.

The IgG or IgM ELISA test (Imunotoxo Bioclin-Quibasa S/A®) (dependent variable) was considered positive when the optical density was higher than the cutoff point calculated for each reading according to the manufacturer's instructions. This kit included four calibrator concentrations that determined the calibration curve to calculate

sample values. The cutoff was 9.0IU/ml with absorbance at 450nm. The independent variables obtained from the questionnaire were grouped as follows: 1) sociodemographic variables (ethnicity, education, marital status, age, family income, place of residence, work, number of pregnancies, number of people per household, and water source); 2) dietary habits (meat intake, raw or undercooked meat intake, using an unwashed knife or cutting board before preparing vegetables or other food, and intake of fruits and vegetables, raw milk, artisanal cheese or sausage); 3) behavioral habits (if the participant prepares food, has pets such as cats or dogs, practices gardening, swims, or owns a vegetable garden).

The database was set up in Epi-Info 3.3.2. We calculated the frequency distribution of variables to build the profile of the participants in the study. We estimated the prevalence of toxoplasmosis by dividing the number of cases by the total positive ELISA serological tests, using a confidence interval of 95% (95% CI)[10].

All the variables were dichotomized (yes or no) except the following variables: family income, which was stratified into five brackets; education level, which was stratified into two levels; and number of household inhabitants, which was stratified into three brackets. The chi-square test was calculated with a 95% CI among the subgroups formed from each variable.

The determination of the independent effect between the selected variables and positive ELISA results was performed using a logistic multivariate model. We estimated the adjusted odds ratio (OR) with a 95% CI between subgroups formed from each variable.

For the dichotomized variables, the adjusted OR was calculated using the stratified analysis of Mantel-Haenszel (OR-MH) for age and education level. In the OR-MH analysis, the homogeneity test yielded a p value>0.05; in other words, there was no significant difference between the frequencies within the subgroups of the confounding variables. Therefore, the adjusted OR could be validated. For the predictive variables, an adjusted OR calculation was performed using binary logistic regression (with IBM® SPSS® Statistics, v. 20.0) during analyses that included more than one category.

The measures of association obtained by a study of prevalence and odds ratio allow us to estimate the magnitude of an association between exposure and disease. These statistics also indicate the probability of developing the disease in the group of exposed individuals compared to unexposed individuals[10,11]. The IgM-positive pregnant women were treated with spiramicin throughout pregnancy.

### Ethical considerations

The study was approved by the Ethics Committee in Research of the University Center (UNIRG - *Universidade Regional de Gurupi*) in Gurupi, Tocantins (protocol number 394846).

## RESULTS

A total of 501 samples were collected; however, 14 (2.9%) pregnant women decided to withdraw from the study or had errors in their questionnaire responses and therefore were excluded from the research. Only patients who correctly answered the questionnaire and had uncomplicated serological testing results were considered in this research, which resulted in a total of 487 women.

From the selected sample (n=487), 333 (68.4%) pregnant women were positive for IgG antibodies against *T. gondii* (CI 95%: 63.37 to 73.37), and 26 (5.3%) pregnant women (CI 95%: 5.07 to 5.61) were positive for IgM antibodies against this parasite. All IgM positive women were also IgG positive.

The sociodemographic characteristics associated with the risk of toxoplasmosis infection (p<0.05) included the following: an education level lower than 8 years, working outside the home, or a family income equal to or lower than two minimum wages **(Table 1)**.

**TABLE 1 -** Uni- and multivariate analysis of sociodemographic and behavioral characteristics related to *Toxoplasma gondii* seropositivity in 487 pregnant women treated in basic health units in Gurupi, State of Tocantins, Brazil.

| Epidemiological factors | | Number positive/total | Univariate model | | Adjusted model | |
|---|---|---|---|---|---|---|
| | | | crude OR | CI95% | adjusted OR | CI 95% |
| Age | < 30 | 241/364 | 0.670 | 0.408-1.100 | 1.130 | 0.654-1.954 |
| | > 30 | 76/102 | 0.879 | 0.543-1.032 | 5.273 | 1.166-23.844 |
| Education level (years) | ≤ 8 | 94/121 | 1.893 | 1.171-3.062 | 6.612 | 1.450-30.144 |
| | > 8 | 228/352 | 0.974 | 0.952-0.997 | 1.417 | 0.839-2.394 |

| Epidemiological factors | | Number positive/total | Univariate model | | Adjusted model | |
|---|---|---|---|---|---|---|
| | | | crude OR | CI95% | adjusted OR | CI 95% |
| Residence | rural area | 16/20 | 1 | | 1.449 | 0.155-13.594 |
| | urban area | 297/439 | 1.912 | 0.628-5.825 | 2.063 | 0.571-7.456 |
| Marital status | single | 63/90 | 0.984 | 0.954-1.015 | - | |
| | married | 126/177 | 0.992 | 0.977-1.008 | - | |
| | stable union | 127/198 | 0.992 | 0.977-1.008 | - | |
| | divorced | 2/4 | 0.667 | 0.300-1.484 | - | |
| | widow | 1/1 | 1 | | - | |
| Work outside home (profession) | yes | 113/179 | 1.596 | 1.059-2.408 | 1.604 | 1.015-2.536 |
| | no | 202/285 | 1 | | 1.566 | 0.620-3.958 |
| Number of pregnancies | 1 | 122/186 | 0.663 | 0.348-1.263 | 0.663 | 0.348-1.263 |
| | 2 | 93/143 | 0.647 | 0.333-1.258 | 0.656 | 0.367-1.173 |
| | 3 | 61/82 | 1.010 | 0.475-2.149 | 1.025 | 0.648-1.620 |
| | ≥4 | 46/62 | 1 | | 1 | |
| Family income | < 1 MW | 108/148 | 2.700 | 1.891-8.182 | 2.898 | 1.158-7.249 |
| | between 1 and 2 MW | 112/161 | 2.286 | 0.761-6.867 | 2.700 | 1.891-8.182 |
| | between 3 and 4 MW | 70/111 | 1.707 | 0.559-5.213 | 1.409 | 0.642-3.093 |
| | between 5 and 6 MW | 23/35 | 1.917 | 0.544-6.749 | 1.581 | 0.931-2.685 |
| | ≥ 7 MW | 7/14 | 1 | | 1 | |
| Number of people living in the residence | 1-5 | 267/395 | 1 | | 1 | |
| | 6-10 | 23/35 | 0.919 | 0.443-1.905 | 0.522 | 0.030-9.095 |
| | >10 | 1/2 | 0.479 | 0.030-7.726 | 0.479 | 0.030-7.726 |
| Source of water | treated water | 259/386 | 0.777 | 0.464-1.301 | 1.413 | 0.781-2.557 |
| | untreated water | 63/87 | 1.300 | 0.770-2.197 | 1.033 | 0.335-3.188 |

**OR:** odds ratio; **CI95%:** confidence interval 95%; **MW:** minimum wage.

The dietary habits that were associated with toxoplasmosis seropositivity ($p<0.05$) included the following: meat intake, cutting raw meat without washing the cutting board before processing vegetables, *in natura* milk intake and frequent vegetable intake **(**Table 2).

**TABLE 2 -** Uni- and multivariate analysis of dietary habits related to *Toxoplasma gondii* seropositivity in 487 pregnant women treated in Basic Health Units in Gurupi, State of Tocantins, Brazil.

| Epidemiological factors | | Number positive/total | Univariate model | | Adjusted model | |
|---|---|---|---|---|---|---|
| | | | crude OR | CI95% | adjusted OR | CI 95% |
| Meat intake | yes | 317/464 | 1.725 | 1.057-6.518 | 1.780 | 1.149-4.080 |
| | no | 5/9 | 1 | | 0.240 | 0.169-1.340 |
| Uncooked meat intake | yes | 74/103 | 1.223 | 0.754-1.983 | 1.289 | 0.747-2.224 |
| | no | 240/355 | 1 | | 1.263 | 0.415-3.849 |
| Type of raw meat | beef | 250/358 | 2.315 | 0.894-5.992 | 2.092 | 0.827-5.293 |
| | chicken | 52/77 | 1.200 | 0.735-5.884 | 1.184 | 0.707-1.982 |
| | pork | 9/18 | 1 | | 1 | |
| Raw kibbeh intake | yes | 6/10 | 0.722 | 0.200-2.602 | 0.254 | 0.046-1.409 |
| | no | 264/391 | 1 | | 1.397 | 0.218-1.602 |

| Epidemiological factors | | Number positive/total | Univariate model | | Adjusted model | |
|---|---|---|---|---|---|---|
| | | | crude OR | CI95% | adjusted OR | CI 95% |
| Undercooked barbecue intake | yes | 122/174 | 1.161 | 0.775-1.741 | 0.597 | 0.323-1.105 |
| | no | 198/296 | 1 | | 0.792 | 0.235-2.666 |
| Wash the knife and the cutting board after cutting meat and before preparing vegetables | wash with water | 30/58 | 1 | | 0.757 | 0.437-1.310 |
| | do not wash | 61/82 | 2.711 | 1.326-5.542 | 2.051 | 1.165-3.614 |
| | wash with water and soap | 222/323 | 1.051 | 0.165-3.614 | 1 | |
| Fruit intake | no | 4/7 | 1 | | 1 | |
| | occasionally | 53/84 | 1.282 | 0.269-6.109 | 1.326 | 0.809-2.174 |
| | frequently | 263/379 | 1.700 | 0.375-7.719 | 1.700 | 0.375-7.719 |
| Vegetable intake | no | 10/17 | 1 | | 1 | |
| | occasionally | 53/92 | 0.951 | 0.333-2.720 | 0.581 | 0.216-1.568 |
| | frequently | 258/363 | 1.720 | 1.038-4.639 | 2.051 | 1.368-3.006 |
| Raw milk intake | yes | 185/259 | 1.405 | 0.953-2.071 | 2.422 | 1.014-5.785 |
| | no | 137/214 | 1 | | 1.171 | 0.756-1.815 |
| Fresh cheese | yes | 201/289 | 1.189 | 0.802-1.764 | 1.14 | 0.736-1.768 |
| | no | 121/184 | 1 | | 1.187 | 0.430-3.276 |
| Raw sausage intake | yes | 132/184 | 1.323 | 0.884-1.978 | 1.324 | 0.840-2.087 |
| | no | 190/289 | 1 | | 1.44 | 0.580-3.575 |
| Garbage handling | regular public collection | 75/105 | 1.215 | 0.754-1.956 | 1.358 | 0.783-2.354 |
| | no public collection | 247/367 | 1.667 | 0.664-4.187 | 0.798 | 0.288-2.215 |

**OR:** odds ratio; **CI95%:** 95% confidence interval.

In this study, there were no behavioral characteristics associated with toxoplasmosis seropositivity (**Table 3**). When the variables were analyzed through the logistic regression model for simultaneous analysis, very similar crude and adjusted odds ratios were observed. This shows that the observed effects of different variables were independent, without significant interference from confounding factors.

**TABLE 3 -** Uni- and multivariate analysis of behavioral characteristics related to *Toxoplasma gondii* seropositivity in 487 pregnant women assisted in Basic Health Units in Gurupi, State of Tocantins, Brazil.

| Epidemiological factors | | Number positive/total | Univariate model | | Adjusted model | |
|---|---|---|---|---|---|---|
| | | | Crude OR | CI95% | Adjusted OR | CI 95% |
| Prepare the food themselves | yes | 267/388 | 1.471 | 0.754-2.869 | 1.471 | 0.754-2.869 |
| | sometimes | 30/42 | 1.667 | 0.664-4.187 | 0.883 | 0.437-1.783 |
| | no | 24/40 | 1 | | | |
| Cats as pets | yes | 48/70 | 1.039 | 0.601-1.794 | 0.884 | 0.486-1.607 |
| | no | 271/400 | 1 | | 4.762 | 0.588-38.574 |
| Dogs as pets | yes | 194/290 | 0.866 | 0.580-1.294 | 0.841 | 0.538-1.316 |
| | no | 126/180 | 1 | | 0.907 | 0.347-2.369 |
| Gardening practice | yes | 65/102 | 0.775 | 0.489-1.229 | 0.638 | 0.373-1.092 |
| | no | 256/369 | 1 | | 1.125 | 0.430-2.942 |
| Swimming practice | yes | 158/240 | 0.811 | 0.550-1.195 | 0.529 | 0.215-1.300 |
| | no | 164/233 | 1 | | 0.946 | 0.612-1.462 |
| The usual place to swim | lake | 2/2 | 1 | | - | |
| | dam | 11/20 | 0.615 | 0.244-1.551 | - | |
| | river | 145/218 | 1 | | - | |

**OR:** odds ratio; **CI95%:** 95% confidence interval.

## DISCUSSION

The prevalence of toxoplasmosis in the present survey is similar to that found in different regions of Brazil, where several epidemiological surveys conducted in pregnant women have shown a rate of approximately 50% to 91%[12,13].

Determining the prevalence of toxoplasmosis associated with prenatal care in pregnant women is important in order to ensure the adoption of prophylactic measures in seronegative patients, to establish an early diagnosis of acute toxoplasmosis in pregnant women, and to employ proper therapy with the goal of reducing the risk of adverse events in patients where fetal seroconversion may take place[14].

The sociodemographic characteristics associated with toxoplasmosis risk included the following: an education level lower than 8 years, working outside the home, and a family income equal to or less than two minimum wages. A maternal education level of greater than 8 years was associated with a lower risk of toxoplasmosis seropositivity (OR: 0.657; CI: 0.243-0.983). This inverse association supports the hypothesis that education decreases the exposure risk due to the adoption of more appropriate hygiene habits, especially in terms of food preparation. An education level of less than 8 years was a risk factor for toxoplasmosis seropositivity (OR: 6.612; CI: 1.450-30.144). These results show that pregnant women with less education had a 6.6-fold higher chance of toxoplasmosis infection. Though some literature studies, such as the description by Cook[15], showed a high prevalence of toxoplasmosis in women with higher education levels and a better socioeconomic status, it is known that low education and socioeconomic status are related to living conditions with an increased risk of contamination. Among these factors, it is important to highlight lack of sanitation, less hygienic habits and a lack of knowledge regarding primary prophylactic measures. The mechanisms of contamination may, however, be different amongst women of different education levels. It is possible that women with less education are exposed at an earlier age, even in childhood, and are more likely to be seropositive by the time they reach reproductive age than more highly educated women. Therefore, more highly educated women may have a greater risk of congenital toxoplasmosis.

The association between toxoplasmosis seropositivity and age is in agreement with previous studies that presented a similar analysis of pregnant women, women of reproductive age and the general population[16]. In most of the published studies, the increase in seropositivity rates occurs after 31 years of age. This may be explained by the longer period of exposure to risk factors[17–19].

In this study, there was a significant difference between age and the incidence of toxoplasmosis in pregnant women, and there was a higher frequency of infection in patients greater than 30 years of age[20,21].

In this study, the risk of *T. gondii* infection was twice as high when pregnant women used unwashed knives and cutting boards after cutting uncooked meat. These results are similar to those of Kapperud[22] in Norway, Guedes[23] in Rio de Janeiro and Ertug[18] in Turkey, who all described a significant association between toxoplasmosis in pregnant women and poor hygiene habits during meal preparation. This epidemiological variable is very difficult to avoid because it is related to the preparation of food for family consumption. Poor hand washing habits and inadequate household utensil hygiene after contact with raw meat can result in the ingestion of bradyzoite cysts present in contaminated food. An effective way to avoid infection is to wash knives and cutting boards after cutting meat or to use different knives to cut meat and other types of food[18].

Regarding the consumption of meat, most published studies have found a direct correlation between meat consumption and *Toxoplasma* infection; this relationship was also found in the present study (OR: 1.780; IC: 1.149-4.080). Among animals, *T. gondii* has been found encysted in the tissues of pigs, sheep and goats more frequently than in the tissues of other domestic animals. Viable *T. gondii* cysts are rarely found in beef. To date, the role played by beef in the epidemiology of *T. gondii* infection is uncertain[24].

In a multicenter European study, Cook[15] determined that the main risk factor for infection in pregnant women was the consumption of raw or undercooked meat[15]. The consumption of this type of food at least once a month increased the risk of acute toxoplasmosis by three times[25].

Regarding the intake of raw vegetables and *in natura* milk by pregnant women, the findings of this study are similar to other studies performed in Brazil and abroad in which it was shown that these two variables are important for toxoplasmosis transmission in women susceptible to infection during pregnancy, as they are directly related to the food preferences of the population (OR: 2.051; CI: 1.368-3.006) and (OR: 2.422; IC: 1.014-5.785), respectively). In Brazil, the results of studies that are in agreement with this work were performed by Brisighelli-Neto[26] in Bragança Paulista (SP), Avelino[27] in Goiânia, Spalding[19] in Alto Uruguai and Oliveira-Bahia[21] in Campos dos Goytacazes (RJ), who found an association between the seropositivity of pregnant women and the intake of raw vegetables. The results obtained by Kapperud[22] in Norway, Cook[15] in Europe and Jones[28] in the United States are also in agreement with the present work. The high consumption of vegetables *in natura* represents a risk of infection. If proper hygiene is lacking, ingestion of oocysts may occur.

Consuming unpasteurized raw milk does not seem to be a common dietary habit, as most of the urban population consumes pasteurized milk. However, the consumption of raw milk in rural areas is common. In this research,

most of the women studied were from urban areas, and a significant association was still found between the consumption of raw milk and infection[29,30].

The presence of pets was not associated with the seropositivity of pregnant women in this study, in spite of the literature determining that cats may disperse oocysts in the environment and that dogs may carry the infective form of the parasite adhered to their fur and thus be involved in mechanical transmission of the parasite[3,31].

According to Cook[15], the results of the study of risk factors for *T. gondii* infection in pregnant women cannot be generalized to countries with different climates and dietary habits. The authors recommend further investigation in order to identify the main risk factors in different populations and to prioritize counseling for pregnant women to prevent acute infection[15]. Several studies demonstrate variability in the prevalence of seropositivity in pregnant women according to different geographic regions, climates, cultural factors and dietary habits[3,12,16].

The present research showed that the study population had been exposed to *T. gondii* infection, as 68.4% of pregnant patients were seropositive for the infective agent. There is also a risk of maternal transmission, as 32.2% of mothers were susceptible to *T. gondii* infection (seronegative). Therefore, prevention of toxoplasmosis is of vital importance because acute infection during pregnancy results in a higher risk of adverse consequences for fetuses and newborns[8].

Knowledge of the risk factors and lifestyle habits that often facilitate infection by *T. gondii* is important for planning educational programs to reduce the incidence of toxoplasmosis during pregnancy, with an emphasis on prevention of infection transmission.

The primary preventive measures against *Toxoplasma* infection are highlighted below. These measures are characterized by education and public health programs that are directed toward pregnant women through campaigns, lectures, pamphlets and educational programs regarding the avoidance of contact with potentially contaminated materials such as cat feces and raw or undercooked meat. It also emphasizes the use of gloves when handling dirt. When this education occurs during the prenatal period, a reduction of 63% in gestational infection may result[8].

It is necessary to organize health services to improve the health and welfare of socially excluded populations; in other words, we need to focus on early identification of pregnant women at risk for *T. gondii* infection and offer them quality prenatal services, according to the Ministry of Health, in order to ensure proper treatment of the pregnant woman.

In conclusion, this study offers important contributions to the understanding of risk factors for congenital toxoplasmosis in Tocantins. The most commonly affected women had lower educational levels, indicating the importance of primary prevention measures in this population. The association between raw meat and *in natura* milk intake with toxoplasmosis seropositivity may be interpreted as a warning for the sanitary authorities to increase and improve meat and milk inspection. Another factor to highlight is the risk of oocyst ingestion with raw vegetables, which is related to poor hygiene habits during food preparation. Measures should be taken to improve sanitary conditions and educate the population regarding *Toxoplasma* infection.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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***Address to:*** Dr. Marcos Gontijo da Silva. Av. Rio de Janeiro, s/n, Centro, 77435-100 Gurupi, TO, Brasil. **Phone:** 55 63 8438-1391. **e-mail:** gontijobio@yahoo.com.br



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**Caixa Postal 118**
**38001-970 Uberaba MG Brazil**
**Tel.: +55 34 3318-5287**
**Fax: +55 34 3318-5279**

e-Mail

rsbmt@rsbmt.uftm.edu.br