



BRIEF COMMUNICATION

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## Seroprevalence and risk factors of *Toxoplasma gondii* infection among open market workers in Ananindeua, Pará, Brazil

### *Soroprevalência e fatores de risco para infecção por Toxoplasma gondii entre trabalhadores do feiras livres em Ananindeua, Pará, Brasil*

**ABSTRACT:** Herein, the presence of antibodies against *Toxoplasma gondii* in food handlers from open markets, and potential risk factors for exposure to the agent, were investigated. A total of 169 food handlers were included in the study. Blood samples were collected and evaluated by enzyme-linked immunosorbent assay. In total, 89.3% (151/169) of the samples were positive for anti-*T. gondii* antibodies. Moreover, “sex” and “drink water or juice of unknown origin” were found to be significantly associated with the risk of infection, whereas “keeping a cat” was not. Food handlers in free markets have high frequency of anti-*T. gondii* antibodies.

**RESUMO:** Demonstramos a presença de anticorpos contra *Toxoplasma gondii* em manipuladores de alimentos de feiras livres e investigamos os fatores de risco potenciais para exposição ao agente. Um total de 169 manipuladores de alimentos foram incluídos no estudo. Amostras de sangue foram coletadas e avaliadas por ensaio imunoenzimático (ELISA). No total, 89,3% (151/169) das amostras foram positivas para anticorpos anti-*T. gondii*. Além disso, as variáveis “sexo” e “beber água ou suco de origem desconhecida” foram significativamente associados ao risco de infecção, ao passo que “ter um gato” não estava. Os manipuladores de alimentos em mercados livres apresentam alta frequência de anticorpos anti-*T. gondii*.

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## 1 Introduction

Toxoplasmosis, caused by *Toxoplasma gondii*, is a cosmopolitan protozoonosis and is among the most prevalent parasitic infections in animals and humans. Felids act as the definitive host of *T. gondii*, whereas birds and mammals are the intermediate hosts (Hill & Dubey, 2002). The main forms of transmission of this protozoan are attributed to the ingestion of food contaminated with cystic forms, such as raw or undercooked meat (Vicente *et al.*, 2014), or the ingestion of the infectious oocyst form through the consumption of contaminated water, fruits, and vegetables (Dubey, 2004).

Epidemiological surveys carried out in the last decades have demonstrate that parasitic infections can have a quite variable incidence globally, ranging from less than 1% up to 77%. However, these differences vary according to the geographic region, as well as behavioral and eating habits (Spalding *et al.*, 2005; Djurković *et al.*, 2019).

Brazil has a tropical climate, with a favorable temperature for the sporulation of oocysts, in addition to varied eating habits in different regions, which can create the optimal setting for *T. gondii* infection.

In Brazil, the seroprevalence of anti-*T. gondii* antibodies in humans varies from 40% to 80%, based on data obtained from studies that have investigated different populations exposed to the agent (Coêlho *et al.*, 2006; Vicente *et al.*, 2014; Vitaliano *et al.*, 2015). However, no report is available on the seroprevalence of anti-*T. gondii* antibodies in food handlers from open markets. Such individuals may have an increased risk of infection, as they are in daily contact with animal and vegetable products, besides staying at the markets for long periods during the day and being forced to eat at or near the workplace. Furthermore, these workers usually do not have any knowledge of the sanitary quality of the food, market, and/or the source of the drinking water.

Concerns regarding the quality of food, especially how it is handled, exposed for sale, and preserved, have attracted great attention, since the risk of contamination is high for its consumers and the marketer itself, who is also a consumer (Silva *et al.*, 2011). Consumers can be exposed to the cystic forms of the parasite contained in animal tissue or oocysts contained in vegetables when handling them, in particular when preventive measures are not adopted (Braga Filho *et al.*, 2010).

In light of the above-mentioned issues, this study aimed to estimate the seroprevalence of antibodies against *T. gondii* in food handlers in open markets in the municipality of Ananindeua, PA, Brazil, and to determine the potential risk factors associated with *T. gondii* infection, such as hygienic and sanitary habits.

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## 2 Materials and Methods

The project was approved by the Human Research Ethics Committee of the Evandro Chagas Institute

(approval number 0017/06; CAAE: 0022.0.072.00-06). The study was carried out in five open markets in the municipality of Ananindeua, PA, selected for convenience. A total of 169 food handlers from open markets participated, including snack sellers, vegetable vendors, and butchers, who did not have symptoms of the disease.

Blood sample collection was carried out after the volunteers provided signed informed consent. Concomitant with the sampling, all volunteers answered an epidemiological questionnaire that aimed to gather information on environmental variables and identify potential clinical and epidemiological factors associated with toxoplasmosis. Blood samples (5 mL) were obtained aseptically by venipuncture in vacuum tubes without anticoagulant. The serum was separated by centrifugation at 2,500 rpm for 10 min and stored at  $-20\text{ }^{\circ}\text{C}$  until serological tests were performed. The serum was assessed using indirect enzyme-linked immunosorbent assay (ELISA) with commercial kits (Sym Toxo G 96; Symbiosis Diagnostica, Leme, SP, Brasil) to detect anti-*T. gondii* antibodies, IgG (indirect method), and IgM (immunocapture), following the manufacturer's recommendations.

For analysis of the questionnaires, a database was created in the statistical program IBM SPSS Statistics (v. 20.0; IBM Corp., Armonk, NY, USA). The data were analyzed using Pearson's chi-square test or Fisher's exact test, and binary logistic regression, with a significance level of  $p \leq 0.05$ .

## 3 Results and Discussion

Of the 169 serum samples tested, 89.3% (151/169) were reactive for anti-*T. gondii* antibodies, demonstrating a high incidence of these antibodies among the food handlers, who had probably been previously infected with this protozoan. Regarding the quantification of the antibodies, only 1.30% (2/151) of the samples were positive for both IgG and IgM, whereas 98.7% (146/151) showed a positive reaction only for IgG.

This is the first seroepidemiological survey that assessed the presence of antibodies against *T. gondii* in food handlers in northern Brazil. Our results revealed a high seroprevalence of antibodies against *T. gondii* among food handlers from open markets in the municipality of Ananindeua, PA, Brazil. This finding is in accordance with data from other studies carried out outside Brazil, wherein seroprevalence in humans was estimated to range from 30% to 70% (Pappas *et al.*, 2009). Although no studies have been carried out specifically in free market workers, this high seroprevalence was already expected, as this population is considered the most susceptible to *T. gondii* infection due to the handling of contaminated food (Alizadeh *et al.*, 2018), similar to farmers, butchers, fruit and vegetable vendors, as well as homemakers.

ELISA data revealed a high frequency of IgG antibody titers, indicative of a chronic infection, whereas only two samples reacted to IgM, which indicates a recent

or acute infection (Sadiqui *et al.*, 2019). Notably, the participant did not report any symptoms compatible with toxoplasmosis, suggesting that the two individuals who presented IgM antibodies were possibly asymptomatic. Regarding the high number of reactions to IgG, it should be considered that *T. gondii* establishes an infection throughout life (Melchor *et al.*, 2019), which justifies the high percentage detected.

When comparing the ELISA results with the information obtained in the epidemiological survey through univariate analyses, it was observed that the variables “sex” and “drink water or juice without knowing the origin” were significantly ( $p \leq 0.05$ ) associated with infection. However, the results of logistic regression analysis suggested that sex (male or female) or the habit of drinking water or juices of unknown origin had no association with the risk of infection by *T. gondii*

(Table 1). The other behavioral variables and habits were not correlated with the probability of exposure to the agent.

Seroprevalence among women was higher compared with men, as demonstrated by the statistical significance of the univariate analysis ( $p < 0.05$ ). However, this trend was no longer significant ( $p > 0.05$ ) in a logistic regression model, which showed that both sexes were equally exposed to the parasite once they have similar eating and behavioral habits, corroborating results obtained in another population group (Mereze *et al.*, 2019).

Analysis of the relationship between the high seroprevalence of anti-*T. gondii* antibodies and education levels of the participants failed to reveal a significant association ( $p = 0.57$ ). In contrast, Mereze *et al.* (2019) reported that illiteracy was a significant risk factor for

**Table 1.** Analysis of variables associated with anti-*T. gondii* antibody seroreactivity in free market workers.

**Tabela 1.** Análise das variáveis associadas à sororeatividade de anticorpos anti-*T. gondii* em trabalhadores de feiras livres de Ananindeua, Pará.

Variables	Category	Total	Univariate Analysis		Logistic Regression		
			Reagents N (%)	P-value (X <sup>2</sup> )	Odds Ratio	IC <sup>(1)</sup> (95%)	P-value
Sex	Male	86	73 (84.88)	0.05**	0.360	0.112-1.060	0.64
	Female	83	78 (93.98)	0.05**	0.360	0.112-1.060	0.64
Scholarity*	Incomplete elementary school	68	62 (91.18)	0.57	1.590	0.288-8.775	0.63
	Complete elementary school	15	13 (86.67)		1.216	0.370-3.999	
	High school	57	51 (89.47)		5.167	0.406-65.67	
Do you drink water or juice without knowing the origin?*	Yes	110	102 (92.73)	0.04**	2.256	0.986-7.156	0.05**
	No	58	48 (82.76)				
Do you have treated water at home?	Yes	105	91 (87.50)	0.222	0.491	0.154-1.567	0.23
	No	57	53 (92.98)				
Wash fruits and vegetables before eating?*	Yes	152	134 (88.16)	0.145	0.00	-	0.99
	No	16	16 (100.0)				
Eat raw salad in restaurant?*	Yes	72	66 (91.67)	0.388	0.636	0.227-1.485	0.39
	No	97	89 (91.75)				
Eat sandwiches with vegetables on the streets?	Yes	97	89 (91.75)	0.240	1.794	0.670-4.803	0.24
	No	72	62 (86.11)				
Eat rare meat?*	Yes	38	36 (94.74)	0.259	2.348	0.512-10.75	0.27
	No	130	115 (88.46)				
Wears gloves while gradining?*	Yes	13	12 (92.31)	0.988	1.017	0.109-9.504	0.98
	No	64	59 (92.19)				
Has knowledge about zoonoses?	Yes	100	88 (88.0)	0.494	1.430	0.510-4.019	0.45
	No	69	63 (91.30)				

<sup>(1)</sup>CI: Confidence Interval

\*Valid data only

\*\*Statistical significance

<sup>(1)</sup>IC: Intervalo de Confiança

\* Apenas dados válidos

\*\*Significância estatística

infection, in a study in which the subjects were literate.

It is recognized that felids can excrete *T. gondii* oocysts into the environment, thus generating environmental contamination that can reach water supply sources and the soil (Alizadeh *et al.*, 2018). Accordingly, a possible relationship between water use and risk of infection was assessed by questioning the following variables: “drink water or juice without knowing the source,” “have water treated at home,” “wash fruits and vegetables before eating,” “eat raw salad in restaurants,” and “eat sandwiches with raw vegetables from the streets”.

In the present study, the intake of water or juice without knowing its origin presented itself as a risk factor in the univariate analysis and logistic regression ( $p < 0.05$ ), suggesting that the subjects who claimed to have this behavior were 2.256-times more likely to become infected with the agent compared with those who did not have this behavior. Nevertheless, the handlers who reported consuming raw salad in restaurants and eating sandwiches with raw vegetables from the streets did not have a significantly higher risk of infection; however, having treated water at home and washing fruit before consuming it proved to be a protective factor against *T. gondii* infection (*Odd Ratio* < 1).

Regarding possible environmental contamination by oocysts, food handlers were asked if they used gloves during gardening, to which the vast majority answered “No” (92.1%). However, the fact that they used personal

protective equipment at work had no effect on the results (Table 1).

Although the ingestion of raw or undercooked meat is significantly associated with infection as an important route of transmission of *T. gondii* to humans (Belluco *et al.*, 2018), no statistical association of this variable with the incidence of *T. gondii* antibodies was observed (Table 1). This is probably because the meat consumed did not contain the infectious form of the parasite. However, preventive measures before meat consumption should be adopted, such as cooking at 67 °C or freezing for two days at -20 °C to inactivate the parasite (Alizadeh *et al.*, 2018).

In the present study, 40.8% of the participants reported not having knowledge about zoonoses, highlighting the need for educational and health campaigns for the target population. A study carried out by Braga Filho *et al.* (2010), aiming to evaluate the degree of knowledge about toxoplasmosis in two municipalities in the state of Pará, demonstrated that the population lacked information regarding the mechanisms of transmission and prevention of the agent (80%), corroborating the findings of the present study. This may have favored the high frequency of antibodies against *T. gondii* here described.

The behavioral aspects of subjects who had cats as pets was also evaluated, revealing that having a cat was not related to the risk of infection by *T. gondii* (Table 2) regardless of the fact that felines are the definitive host for this parasite. Moreover, only the variables “cat defecates

**Table 2.** Distribution of individuals reactive to the ELISA, according to information extracted from the epidemiological questionnaire in relation to possible risk factors involving the cat.

**Tabela 2.** Distribuição de indivíduos reagentes ao Ensaio Imunoenzimático (ELISA), de acordo com informações extraídas do questionário epidemiológico em relação a possíveis fatores de risco envolvendo o gato.

Variables	Category	Total	Univariate Analysis		Logistic Regression		
			Reagents N (%)	P-value (X <sup>2</sup> )	Odds Ratio	IC <sup>(1)</sup> (95%)	P-value
Do you have contact with a cat?	Yes	49	45 (91.84)	0.50	1.486	0.460-4.762	0.50
	No	120	106 (88.33)				
Do you have a cat?	Yes	48	44 (91.67)	0.53	1.439	0.449-4.616	0.54
	No	121	107 (88.43)				
Cat defecates in the litter box?*	Yes	06	02 (33.33)	0.001*	000	-	0.99
	No	40	40 (100.0)				
Is the cat in contact with vegetable gardens?*	Yes	01	01 (100.0)	0.76	1404	-	1.00
	No	28	26 (92.86)				
Cat eats raw meat?*	Yes	07	06 (85.71)	0.58	0.514	0.046-5.802	0.59
	No	38	35 (92.11)				
Cat hunts rodents?*	Yes	38	35 (92.11)	0.67	1.667	0.151-18.45	0.67
	No	08	07 (87.50)				
Eat rare meat?*	Yes	38	36 (94.74)	0.259	2.348	0.512-10.75	0.27
	No	130	115 (88.46)				

<sup>(1)</sup>CI: Confidence Interval

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in the litter box” was found to represent a risk of infection in the univariate analysis; however, no significant association was identified in the logistic regression analysis ( $p = 0.99$ ). Nonetheless, it should be noted that, in the same analysis, an *Odd Ratio*  $< 1$  was observed, which suggests that the fact that cats have a litter box confers a protective factor for their guardians against *T. gondii* infection. Other variables such as “cat has contact with vegetable gardens,” “cat eats raw meat,” and “cat hunts rodents” were not statistically significant, although the involvement of *T. gondii* environmental contamination by eliminating oocysts in cat feces has been recognized (Alizadeh *et al.*, 2018). The factor of eating raw meat can pose a risk to these animals, as the agent can affect a wide range of intermediate hosts, and rodents play an important role in the prey–predator relationship, thereby contributing to feline infection (Melchor *et al.*, 2019).

Lack of a statistical correlation between most of the factors presented in the epidemiological questionnaire with the serological results was also reported by Mereze *et al.* (2019) in Paraná. According to the authors, this was probably be due to the use of subcategories in the studied variables, which granted very wide confidence intervals, thereby interfering with the statistical analysis results, even in the presence of an adequate number of samples and a low number of non-reactive results. This is similar to the statistical findings of the present study.

## 4 Conclusion

In conclusion, there is a high seroprevalence of anti-*T. gondii* antibodies among food handlers from open markets in the municipality of Ananindeua, PA, Brazil, with the ingestion of water or juice without knowing its origin being an important risk factor for the ingestion of oocysts, and the habit of pet cats defecating in a sandbox acting as a protective factor against infection of their guardians.

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