

Prevalence and risk factors of toxoplasmosis among adults in a small Brazilian city

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Abstract

Introduction: The prevalence of *Toxoplasma gondii* infection varies markedly among different populations, especially depending on factors related to socioeconomic development and eating habits. Cássia dos Coqueiros is a small city in Brazil with rural characteristics and increased risk factors traditionally associated with *T. gondii* infection. **Methods:** We carried out a cross-sectional study involving 970 inhabitants aged 18 years or more, selected from patients of the local health unit and home visits in urban and rural areas. Each participant completed a survey with questions regarding demographic, socioeconomic, and risk factors for toxoplasmosis. Blood samples from participants were tested for presence of IgG and IgM antibodies against *T. gondii* using a chemiluminescent microparticle immunoassay. **Results:** The prevalence of IgG and IgM antibodies was 62.3% and 2.5%, respectively. Variables that proved to be independent predictors of infection were age, low levels of education, and previous diagnosis of toxoplasmosis. **Conclusions:** The high prevalence of toxoplasmosis serological markers in this adult population highlights the need to promote preventive practices, especially those directed toward women of childbearing age, in this part of Brazil.

Keywords: Toxoplasmosis. Population survey. Prevalence. Risk factors. Rural.

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan that is distributed worldwide in felids, especially cats, which are its definitive host¹. The infection is estimated to affect approximately one-third of the Earth's population, including several mammals, as well as domestic and wild birds¹. Human infection is usually asymptomatic, but it may manifest clinically in different ways: febrile, generalized lymphadenopathy, when occurring in acute infection of adults and children; ocular and congenital toxoplasmosis, when primary infection occurs in pregnant women and the fetus; and meningoencephalitis owing to reactivation of protozoan cysts in patients with severe immunosuppression, such as people living with HIV/AIDS or those using drugs that significantly interfere with the immune response¹.

Epidemiological characteristics of *T. gondii* infection vary around the world and may even differ within the same country¹. Several conditions can influence the results of studies on the prevalence of infection, especially related to characteristics of individuals included in the study (general populations or specific groups, such as pregnant women) and their age groups. In fact, the high potential of congenital infection to cause severe deformities in newborns has helped yield a considerable portion of the epidemiological studies on toxoplasmosis, which refer to populations of women of childbearing age or pregnant women²⁻⁶.

The main factors associated with the great variability in the distribution of *T. gondii* infection are socioeconomic, environmental, and cultural differences, as well as those related to living and eating habits of the residents of a given region¹. Thus, prevalence ranging from 8.2% to 63.2% has been described in Europe, according to the studied region, city, and population; this phenomenon has also been reported in different Asian countries⁶. In the Americas, the variation ranges from the 11.0% described in some regions of the United States⁷ to values approaching 80% in specific areas of South American countries⁶. In Brazil, a review of different investigations shows the same pattern of wide variability in the distribution of infection, exemplified by values of 7.3% among women of childbearing

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age in Brasília, to 77.5% and 79.0% among pregnant women⁶ and blood donors⁸ in Recife, respectively.

The aim of this study was to assess the prevalence of antibodies against *T. gondii* and risk factors for infection in an adult population representative of the residents of a small city with rural characteristics, located in the Southeast region of Brazil.

METHODS

To carry out different epidemiological investigations proposed within the scope of the Ribeirão Preto Medical School (FMRP), between 2010 and 2013, we collected 30-mL blood samples from 1,150 residents aged 18 years or more in Cássia dos Coqueiros, which is located 80 km from Ribeirão Preto and has an estimated population of 2,591 inhabitants. All samples were obtained by cubital venipuncture performed by the nursing staff of the Pedreira de Freitas Community Social Medical Center (CMSCPF) of the FMRP using disposable materials. Samples were centrifuged in the Laboratory of Serology of the Clinics Hospital of the Ribeirão Preto Medical School (HCFMRP) and the serum samples were maintained at -20°C in properly labeled Eppendorf tubes. Initially, all individuals who attended the CMSCPF for any complaint and fulfilled the inclusion criteria were invited to participate: current resident of the city, 18 years of age or older, and having signed an informed consent form. Subsequently, the active search phase for potential participants was carried out through visits to all households in urban and rural areas, where residents are continuously encouraged to participate in research by Community Health Agents of the Family Health Program (PSF).

Between 2011 and 2013, all individuals who had undergone blood sample collection were invited to complete a specific survey that included questions on demographic and socioeconomic information as well as risk factors for different infections. In relation to toxoplasmosis, we included questions regarding the presence of felines and other domestic animals in the home, manual contact with soil and home gardens, habitual consumption of undercooked meat (pork or mutton), habitual consumption of raw vegetables, previous diagnosis of toxoplasmosis, previous diagnosis of eye diseases, and elimination of proglottids in the feces. Classification of economic strata was according to the Brazilian Economic Classification Criterion⁹. More than 90% of participants were interviewed by a doctoral candidate whose thesis was focused on viral hepatitis B and C. The other interviews were conducted by a properly trained community health agent. Before survey administration, participants were asked to sign a new informed consent form.

We carried out a cross-sectional study involving 990 participants from among the 1150 individuals for whom we had stored serum samples and a completed survey; the study population corresponded to 50.3% of the estimated number of area residents aged 18 years or more, according to the 2010 Brazilian National Census.

Laboratory tests were performed at the Laboratory of Serology of the HCFMRP and consisted of detecting IgG and IgM antibodies against *T. gondii* by means of a chemiluminescent

microparticle immunoassay (CMIA) using ARCHITECT Toxo IgGTM and ARCHITECT Toxo IgMTM kits, according to the manufacturer's specifications (Abbott, Wiesbaden, Germany).

Samples with inconclusive results were retested at the same laboratory. All patients with positive results for IgG and/or IgM antibodies, as well as 30% of those with negative results, were asked to attend the CMSCPF for an ophthalmological evaluation performed by FMRP specialists. These exams were performed as a joint effort for eight weekends, with up to three requests made to each individual who did not appear on their scheduled date (results of the ophthalmological findings will be published in the future).

The information from the survey and laboratory results were entered into a database using an Excel spreadsheet and then transferred to the SAS 9.4 software package (SAS Institute Inc., Cary, NC, USA). In the initial univariate analysis, possible associations between potential risk factors and the presence of serological markers of toxoplasmosis were tested by calculating the prevalence rates and their respective confidence intervals. Sex and other variables that showed evidence of an association ($p < 0.25$) were included in a log-binomial regression model.

To carry out all the steps that led to conducting this study, three approvals were obtained from the HCFMRP Research Ethics Committee; we received approval to 1) create a human serum bank (HCFMRP process No. 3,915/2010); 2) administer the survey (HCFMRP process No. 24,460/2012); and 3) carry out the toxoplasmosis study (HCRP process No. 13880/2014).

RESULTS

Serological results and complete surveys were obtained from 990 participants with the following results regarding the presence of IgG marker: 617 positive (62.3%), 353 negative (35.7%), and 20 inconclusive (2.0%). IgM marker was present in 25 participants (2.5%) and 24 of them were also found to be positive for IgG. Excluding any inconclusive results, 970 participants were included in the analyses, and 618 (63.7%) of them had IgG and/or IgM antibodies against *T. gondii*.

The data in **Table 1** reveals that a predominance of participants were female (60.3%), inhabitants of urban areas (69.3%), and natives of São Paulo State (74.8%). A high percentage of participants and heads of household had low education levels, with percentage of people with only up to 4 years of school attendance corresponding to 27.2% and 31.6%, respectively. There was a marked predominance of participants from economic strata C (58.4%) and B (31.8%).

A gradient of infection risk was observed according to age, with percentages ranging from 41.0% among individuals under 25 years old to 74.3% among individuals aged 64 years or more (**Table 2**). An opposite phenomenon was observed in relation to the variable of schooling for participants and heads of household, with infection percentages decreasing steadily with increased educational level. Individuals with less than 4 years of schooling showed an infection rate (76.1%) corresponding to double of that observed among people with 15 or more years of school attendance (37.6%). Belonging to lower economic strata (C, D, and E) was also associated with a higher percentage of

TABLE 1: Demographic and socioeconomic variables of the study population in Cássia dos Coqueiros, SP, 2015.

Variable	n	%
Sex		
male	385	39.7
female	585	60.3
Age (years)		
<25	100	10.3
25-34	172	17.7
35-44	183	18.9
45-54	209	21.5
55-64	158	16.3
>64	148	15.3
Area of residence		
urban	672	69.3
rural	298	30.7
State of origin		
São Paulo	726	74.8
other	244	25.2
Schooling (years)		
up to 4	264	27.2
5 to 7	247	25.5
8 to 10	109	11.2
11 to 15	257	26.5
>15	93	9.6
Schooling of the head of household (years)		
up to 4	307	31.6
5 to 7	278	28.7
8 to 10	117	12.1
11 to 15	207	21.3
>15	61	6.3
Economic stratum		
A2	10	1.0
B1 or B2	308	31.8
C1 or C2	566	58.4
D	83	8.6
E	3	0.3

positive results than belonging to strata A and B (66.4% and 58.2%, respectively).

Table 3 shows an association between IgG and IgM markers for toxoplasmosis and the following variables: presence of felines in the home, consumption of undercooked meat, previous diagnosis of eye diseases, previous diagnosis of toxoplasmosis, contact with rodents at home or work, and history of *Taenia* spp. proglottid elimination.

Multivariate analysis (Table 4) showed that the variables age, schooling, and previous diagnosis of toxoplasmosis were independent predictors of presence of IgG antibody against *T. gondii* in the studied population.

DISCUSSION

The predominance of female participants in this study can be explained by the greater access of women to the Health Unit in Cássia dos Coqueiros, and the difficulty in finding men at home during field sample collection. The low percentage of individuals aged up to 25 years is owing to the cut-off point of 18 years adopted for inclusion in this study, a decision taken to avoid the difficulties arising from selection of underage participants and the consequent need to obtain authorization from a parent or guardian. Although this option resulted in greater operational ease of executing the research, it is impossible to know the distribution of infection markers in this younger population, which is a limitation of the study.

TABLE 2: Distribution of positive results for toxoplasmosis according to demographic and socioeconomic variables.

Variable	Total	Positive		PR (95% CI)	p
		n	%		
Sex					
female	585	382	65.3	1.06 (0.96–1.18)	0.21
male	385	236	61.3	Ref.	
Age (years)					
<25	100	41	41.0	Ref.	
25–34	172	103	59.9	1.46 (1.12–1.90)	<0.01
35–44	183	126	68.9	1.67 (1.30–2.17)	<0.01
45–54	209	131	62.7	1.53 (1.18–1.98)	<0.01
55–64	158	107	67.7	1.65 (1.28–2.14)	<0.01
>64	148	110	74.3	1.81 (1.40–2.34)	<0.01
Area of residence					
urban	672	429	63.8	1.01 (0.90–1.16)	0.90
rural	298	189	63.4	Ref.	
State of origin					
São Paulo	726	455	62.7	Ref.	
other	244	163	66.8	1.07 (0.96–1.18)	0.23
Schooling (years)					
up to 4	264	201	76.1	2.02 (1.54–2.65)	<0.01
5 to 7	247	167	67.6	1.79 (1.36–2.37)	<0.01
8 to 10	109	68	62.4	1.66 (1.23–2.23)	<0.01
11 to 15	257	147	57.2	1.52 (1.14–2.01)	<0.01
>15	93	35	37.6	Ref.	
Schooling of the head of household (years)					
up to 4	307	222	72.3	1.76 (1.29–2.40)	<0.01
5 to 7	278	177	63.7	1.55 (1.13–2.13)	<0.01
8 to 10	117	72	61.5	1.50 (1.08–2.09)	<0.01
11 to 15	207	122	58.9	1.44 (1.04–1.98)	<0.01
>15	61	25	41.0	Ref.	
Economic stratum					
A and B	318	185	58.2	Ref.	
C, D, and E	652	433	66.4	1.14 (1.02–1.27)	<0.01

PR: Prevalence rate; CI: Confidence Interval.

The higher number of urban residents in the study largely reflects the spatial distribution of the city's population, which is now mostly located in the urban area. Secondarily, this may also be related to greater ease of access by urban residents to the Health Unit, as well as by researchers to homes in the urban area.

The fact that nearly a quarter of study participants were from states other than São Paulo is primarily because of the proximity of Cássia dos Coqueiros to Minas Gerais State, facilitating the exchange of people between both states. In addition, until recently, Cássia dos Coqueiros attracted large numbers of temporary workers from other states who arrived for the *Brachiaria* grass harvest, some of whom never returned to their states of origin.

The finding that more than half of participants had a maximum of only 7 years' schooling, with almost 30% having completed only 4 years (in a study universe where underage people were not included) shows the low educational level of the studied population. The comparison with heads of household shows a small evolution in the schooling level, although the overlap in the two categories with a non-negligible number of participants should be taken into account.

According to the Brazilian Economic Classification Criterion⁹, there is a large predominance of people belonging to economic strata C1 and C2, followed by B1 and B2, in Cássia dos Coqueiros. There were few people belonging to the lowest strata, D and especially E, among our study participants. Thus,

TABLE 3: Distribution of positive results for toxoplasmosis according to different exposures.

Exposure	Total	Positive		PR (95% CI)	p
		n	%		
Felines at home					
yes	685	451	65.8	1.12 (1.01–1.26)	0.04
no	285	167	58.6	Ref.	
Consumption of undercooked meat					
yes	119	87	73.1	1.17 (1.04–1.32)	0.01
no	851	531	62.4	Ref.	
Consumption of raw vegetables					
yes	927	591	63.8	1.01 (0.80–1.28)	0.90
no	43	27	62.8	Ref.	
Manual contact with soil/garden					
yes	553	351	63.5	Ref.	0.86
no	417	267	64.0	0.99 (0.90–1.09)	
Previous eye disease					
yes	136	102	75.0	1.21 (1.09–1.35)	<0.01
no	834	516	61.9	Ref.	
Previous toxoplasmosis					
yes	26	23	88.5	1.40 (1.21–1.63)	<0.01
no/do not know	944	595	63.0	Ref.	
Contact with rodents					
yes	204	146	71.6	1.16 (1.05–1.29)	<0.01
no/do not know	766	472	61.6	Ref.	
Proglottid elimination					
yes	162	120	74.1	1.20 (1.08–1.34)	<0.01
no/do not know	808	498	61.6	Ref.	

PR: Prevalence rate; CI: Confidence Interval.

TABLE 4: Results of log-binomial model for the association between toxoplasmosis and different covariates.

Variable	PR	CI (95%)	p
Female sex	1.10	0.99–1.21	0.06
Age (in years)			
<25	Ref.		
25–34	1.40	1.07–1.83	0.01
35–44	1.58	1.21–2.05	<0.01
45–54	1.44	1.10–1.87	<0.01
55–64	1.48	1.13–1.95	<0.01
>64	1.67	1.28–2.19	<0.01
Schooling (years)			
up to 4	1.78	1.35–2.35	<0.01
5 to 7	1.64	1.25–2.16	<0.01
8 to 10	1.64	1.22–2.20	<0.01
11 to 15	1.56	1.18–2.06	<0.01
>15	Ref.		
Felines at home	1.10	0.98–1.23	0.08
Consumption of undercooked meat	1.10	0.97–1.24	0.14
Previous diagnosis of eye diseases	1.09	0.98–1.22	0.10
Previous diagnosis of toxoplasmosis	1.34	1.12–1.61	<0.01
<i>Taenia</i> spp proglottid elimination	1.09	0.98–1.21	0.11
Economic stratum	0.98	0.89–1.09	0.76
Contact with rodents	1.06	0.97–1.16	0.23

CI: Confidence Interval; PR: Prevalence rate.

the distribution of inhabitants in the city is concentrated at different levels throughout what could be classified as the mean socioeconomic strata, with a small minority located at the upper and lower ends of the spectrum.

T. gondii infection is widely distributed worldwide and is estimated to affect about one-third of the Earth's population¹, varying markedly among different geographic areas. Thus, diverse prevalence rates have been described, ranging from 16% among women born in Spain² to 84% among individuals with low socioeconomic status in Campos dos Goytacazes, Rio de Janeiro State¹⁰. This large variation has been attributed to socioeconomic conditions in particular as well as to eating and hygiene habits in different populations, as the infection can be transmitted by ingestion of contaminated meat (especially from pigs), contact with cat feces, or via the placenta in an acute infection during pregnancy¹¹. Because of the risk of severe forms of toxoplasmosis in the fetus or newborn, transplacental transmission has great clinical importance and justifies efforts aimed at understanding the immune status of the population; susceptible women who may acquire *T. gondii* infection during pregnancy should be carefully observed and should be targeted for specific treatment to prevent transplacental transmission.

In Cássia dos Coqueiros, we found factors that are traditionally associated with a higher risk of toxoplasmosis infection. For example, in rural areas, there is no public water supply and people rely on private wells. In addition, there are several small pig farms that produce meat for consumption by local residents. There are cats present in approximately 70% of homes, and nearly the entire local population consumes raw vegetables; moreover, the consumption of undercooked meat is not uncommon. There is also a low level of education and the presence of a considerable number of migrants, both factors that have been associated with increased infection risk^{2,4}.

Under these conditions, detection of positive serology (IgG and/or IgM antibodies) for toxoplasmosis in 63.7% of 970 tested adults, with progressively increased values in the highest age groups, is consistent with previous studies in other Brazilian regions that have mainly assessed women of childbearing age or pregnant women. In Natal, Rio Grande do Norte State, the seroprevalence among 190 pregnant women was very similar (66.3%) to that observed in Cássia dos Coqueiros, and also increased proportionally with age³. In other studies involving pregnant women in Londrina, Paraná State, and Divinópolis, Minas Gerais State, the prevalence was respectively 49.2% and 49.6%^{4,5}, showing that about half of participants were susceptible to *T. gondii* infection. However, considerably lower percentages have been described in other investigations involving pregnant women, such as a general prevalence of 21% reported in Albacete, Spain, a value that decreased to 16% among women born in the country².

In the present study, we observed an increased prevalence of *T. gondii* infection according to age, which is a phenomenon that has been widely described in different reports^{3,4,12}, evidencing an accumulated risk proportional to age.

Lower levels of education and low per capita income have been described as being associated with increased risk

of *T. gondii* infection in different studies^{4,10,13}. In our study, multivariate analysis did not show an association between *T. gondii* infection and the economic stratum of participants, contrary to that observed in investigations carried out in other regions such as Natal, Londrina, and Campo dos Goytacazes^{3,4,10}. One of these studies, conducted among 1,436 people, marked differences were found when comparing the economic strata of participants, with extreme values of 23% and 84% among those with the best and worst socioeconomic status, respectively¹⁰. Our observation of variable economic strata, according to the Brazilian Economic Classification Criterion⁹, showed that almost 60% of the inhabitants of Cássia dos Coqueiros belong to category C, with very few people below that level. This homogeneity may have reduced the discriminatory power of this variable regarding classification according to economic and social condition. However, the inverse association of *T. gondii* infection with level of schooling was maintained, which is in agreement with other data in the literature^{3,4} and reinforces the notion of higher risk among people with worse socioeconomic status.

Contamination of water for human consumption by *T. gondii* oocysts^{1,10} suggests possibly higher risks among inhabitants of rural areas who use potentially contaminated wells and mines. However, this was not observed in Cássia dos Coqueiros. The distinction between rural and urban areas is very unclear in the daily life of the city's inhabitants because many urban residents work on small farms and residents from rural areas conduct professional activities in urban areas. In a study carried out in the early 1970s among patients of Clinics Hospital of Ribeirão Preto, a hub city in the same region as Cássia dos Coqueiros, no differences were observed between rural and urban inhabitants, leading the authors to conclude that the characteristics that differentiate these groups are not relevant in the disease transmission process¹⁴.

Although there is no uniformity in the published literature, the results of our study are not in line with most epidemiological investigations in that we did not find an association between *T. gondii* infection and contact with felines^{3-5,11}. In Cássia dos Coqueiros, this is an intriguing fact because almost 70% of study participants reported the presence of cats at home, which increases the risk of oocyst intake by humans.

Although referred to in several previous studies, association between the consumption of undercooked meat and toxoplasmosis was not observed in the present study^{11,15}. In fact, this an unexpected finding because small pig farms are quite common in the rural parts of Cássia dos Coqueiros, with animals usually roaming freely and adequate sanitary practices not always strictly followed. As might be expected, a previous diagnosis of toxoplasmosis proved to be a predictor of a positive serological result.

Although still very high, the prevalence of *T. gondii* infection in Cássia dos Coqueiros was lower than the rates observed among patients from the Ribeirão Preto region in the 1970s, which were over 80%¹⁴. The same decreasing tendency in rates of infection was described in Portugal, where there was a decline from 47% to 22% over three decades (1979 to 2013)¹⁶.

Variables such as urbanization, changes in lifestyle and habits, and better conditions of environmental sanitation and food care appear to influence *T. gondii* infection prevalence among adults¹⁷, which may explain the observed reduction in infection levels over time in our study. Although desirable, lower circulation of the pathogen in a community ultimately increases the number of women of childbearing age who are susceptible to infection and, consequently, lowers the risk of congenital infection. This percentage was equal to 39.9% in the study area, which makes regular testing of pregnant women and adequate guidance on care essential to preventing *T. gondii* infection during the antenatal period.

The present study is subject to at least two limitations. The first is the fact that the selection of participants was not randomly performed; consequently, the possibility of selection bias cannot be completely ruled out, even though the large sample size and inclusion of people from all areas of the municipality very likely reduced this possibility. The second limitation is regarding the non-inclusion of individuals under 18 years old, preventing the generalization of the study results to the entire population. Despite these limitations, this study has clinical relevance as we provide an updated estimate of the percentage of people infected with *T. gondii* in Cássia dos Coqueiros, which will allow comparisons with previous results obtained in the region and serves to measure the impact of preventive measures that may be adopted to reduce the transmission of *T. gondii* in Brazil.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Erratum

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Should read:

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