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Serological study on toxoplasmosis in the Haliti-Pareší community of the Utiariti indigenous territory, Campo Novo do Parecis, Mato Grosso, Brazil

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A B S T R A C T

Toxoplasma gondii is the etiological agent of toxoplasmosis, a widespread zoonosis that affects several homeothermic animals, including humans. This disease causes serious health problems, such that 10% of infected individuals develop clinical manifestations. Some studies on indigenous human populations have indicated variations in seroprevalence from 10.6% to 80.4% in such populations in different regions of Brazil and in other countries like Venezuela and Malaysia. To date, there have been no studies regarding the prevalence of anti-*T. gondii* antibodies in Haliti-Pareší Indians living in Campo Novo do Parecis, Mato Grosso, Brazil. Our objective here was to determine the frequency of occurrence of antibodies against this protozoon in nine Haliti-Pareší villages by correlating seroprevalence with locations and variables. Serodiagnoses were made using the indirect immunofluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) in the Laboratory for Toxoplasmosis and Other Protozoan Diseases of IOC/Fiocruz. It was considered that samples tested positive for *T. gondii* infection if IgG/IgM antibodies against this protozoon were detected through serodiagnosis using either IFAT or ELISA. Among the 293 samples analyzed, 66.9% presented anti-*T. gondii* IgG and 3.4% presented anti-*T. gondii* IgM. It was observed that there were no statistically significant differences in frequency of antibody occurrence among infected individuals, based on sex, schooling or occupation/activities. However, there were statistical differences based on age and villages. The prevalence observed in this study is in agreement with values found in other studies on indigenous populations in Latin America. Like among other such populations, the Haliti-Pareší villages are located close to forests and the individuals have domestic cats as pets, are involved in hunting and farming and consume water directly from water accumulation sources. These factors might cause exposure to *T. gondii* tissue cysts and oocysts.

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1. Introduction

Toxoplasma gondii is an obligate intracellular protozoon with a facultative heteroxenous cycle. It can infect all members of the family Felidae, which are it definitive hosts, and also humans and other animals, which are it intermediate hosts (Baruzzi, 1970; Amendoeira et al., 1999; Hill et al., 2005).

The parasite has three infective forms: tachyzoites, bradyzoites, and sporozoites (Amendoeira et al., 1999; Dubey et al., 1998; Rey, 2011; Lopes and Berto, 1992). It is transmitted horizontally from definitive hosts to intermediate hosts and vice versa (Amendoeira, 1995; Tenter et al., 2000; Montoya and Liesenfeld, 2004; Fajardo et al., 2013). Moreover, the possibility of sexual transmission cannot be ruled out, as shown by Santana et al. (Santana, 2013), who found that *T. gondii* infection can be transmitted sexually from male to female goats.

It has been suggested, from studies conducted in Brazil, that Latin America may have a high prevalence of *T. gondii* infection (Furtado et al., 2013). According to Souza et al. (2010) and Dubey et al. (2012), the seroprevalence in Brazil ranges from 50% to 80%.

In relation to the indigenous populations, it has been found that the prevalence of *Toxoplasma* infection presents geographical variation from 10.6% in Southeast Asia (Hakim et al., 1994) to 80.4% in South America (Amendoeira et al., 2003).

The Haliti-Paresí indigenous villages are located in seven municipalities, all in the middle region of northern Mato Grosso (Terças et al., 2016) and are distributed among nine indigenous lands (Silveira, 2011). The Utiariti indigenous land is divided between the municipalities of Campo Novo do Parecis and Sapezal, and contains nine villages with about 327 individuals in total, all located in Campo Novo do Parecis (Terças, 2016).

Up to the present moment, no reports revealing the health status of these Indians regarding toxoplasmosis have been published. In this light, the objective of the present study was to determine the frequency of occurrence of anti-*T. gondii* IgM and IgG antibodies in individuals of the Haliti-Paresí indigenous people in the Utiariti indigenous territory, in the municipality of Campo Novo do Parecis.

2. Materials and methods

2.1. Study area

The present study was conducted in the Utiariti indigenous land, in nine Haliti-Paresí villages belonging to the municipality of Campo Novo do Parecis. These villages are named Quatro Cachoeiras, Bacaiuval, Bacaval, Seringal/Cabeceira do Seringal, Chapada Azul, Sacre 2, Utiariti, Wazare and Morrim.

Blood collection was performed throughout December 2014 to 2016, and a total of 293 aliquots of serum samples from individuals of the indigenous people were sent to the Laboratory for Toxoplasmosis and Other Protozoan Diseases of IOC/Fiocruz, in Rio de Janeiro.

Only one individual from the village of Morrim was involved in the study because on the day of data collection, there were no other inhabitants present in this area. For this reason, we included this individual in the data set for the village of Bacaiuval, because this village is close to Morrim.

Epidemiological data on each participant, including sex, age, village, schooling and occupation/activities were obtained using a form, which was handed out before blood collection. The subjects were divided into two groups in relation to their occupation/activity: (i) occupational group without risk of *T. gondii* infection; and (ii) occupational group with risk of *T. gondii* infection. The occupations included activities such as agriculture, hunting and food processing (kitchen assistance and cooking).

2.2. Serology

Serological tests were performed between February and July 2017. The 293 serum samples collected were subjected to the indirect immunofluorescence antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA).

The IFAT technique was performed as described by Camargo (1974), following the standard protocol of the Laboratory for Toxoplasmosis and Other Protozoan Diseases, using the following conjugates: anti-human IgM (FITC in goat) (Sigma Aldrich); and anti-human IgG (μ chain-specific; FITC in goat) (Sigma Aldrich). Samples with dilutions greater than or equal to 1:16 were considered seroreactive for anti-*T. gondii* IgM and IgG.

Biolisa toxoplasmosis IgM and Biolisa toxoplasmosis IgG kits (Bioclin) were used to perform immunoenzymatic assays. The procedures and qualitative calculations were done in accordance with the protocol recommended by the manufacturer.

2.3. Statistical analyses

The serological results and the epidemiological variables were analyzed using the GraphPad Prism statistical software. Student's *t*-test was used to evaluate the variable “age”; the chi-square (χ^2) test for the variables “age group” and “schooling”; χ^2 for trend for the variable “activities classified as presenting risk” because there were values smaller than five; partition of χ^2 for the variable “villages”; and Fisher's exact test for the variables “sex”, “occupation without risk”, “occupation with risk”, and “female fertile age”.

All the variables were evaluated in relation to the seroreactivity of the samples. The results were considered statistically significant when $p < 0.05$.

2.4. Ethical considerations

The present study was approved by Research Ethics Committee (CEP) of Júlio Muller Universitary Hospital/Mato Grosso Federal University through report no. 1.904.321, in February 2017, and report no. 1.969.623, in March 2017 by National Commission for Research Ethics (CONEP). These constituted, amendments for inclusion of *T. gondii* infection and other etiological agents that were made to projects that had been approved, respectively, by Research Ethics Committee through report no. 69177, in August 2012 and by the National Commission for Research Ethics through report no. 819.939 in October 2014.

3. Results

Among the total number of serum samples analyzed, over the two years of collection, the frequency of occurrence of anti-*T. gondii* IgG in at least one of the two serological tests was 66.9% ($n = 196$). Eight (4.1%) of the samples that were positive for IgG were also seroreactive for anti-*T. gondii* IgM through only one of the techniques (IFAT).

Twelve samples in total were reactive for anti-*T. gondii* IgM through IFAT. These twelve samples were tested for the presence of rheumatoid factor antibodies: two samples showed cross-reactions and were therefore considered to be non-reactive regarding *Toxoplasma* infection through IFAT. Thus, 3.4% ($n = 10$) of all of the samples were considered seroreactive for anti-*T. gondii* IgM, and two samples were considered to be non-reactive for anti-*T. gondii* IgG. All 293 samples that were subjected to ELISA for anti-*T. gondii* IgM detection tested non-reactive.

Regarding the variable "sex", the percentages of positive samples were 63.8% and 70.2% among males and females, respectively, without any statistically significant differences ($p = 0.265$) between them.

With regard to the variable "age groups" (Table 1) and their ages individually (year by year), there were statistically significant differences among the seroreactive individuals ($\chi^2 = 14.97$; $p = 0.021$ and $t = 1.984$; $p = 0.000$), respectively, with an odds ratio (OR) value of 1.027 (IC 95%, 1.012–1.043).

Among women of childbearing age (from 14 to 40 years), 70% ($n = 49$) seroreactivity was observed. Comparison with seroreactive women outside of this age range (70.4%) showed that there was no statistically significant difference ($p > 0.999$).

Regarding the variable "schooling" (Table 2), it was observed that the frequency of occurrence of anti-*T. gondii* IgG was highest among Haliti-Paresí individuals aged 34 to 94 years who had not attended school at all.

From analysis on the variable "schooling" alone, using the χ^2 test, a statistically significant difference in seropositivity ($\chi^2 = 18.50$; $p = 0.018$) was observed between the educational levels. However, when this variable was analyzed together with age by means of logistic regression, there was no statistical difference in seropositivity between the schooling levels ($p = 0.850$), even with an OR value of 1.014 (IC 95%, 0.875–1.176).

Regarding the frequency of occurrence of seroreactivity for *T. gondii* according to the variable "occupation/activities" among the Haliti-Paresí indigenous population, there was no statistically significant difference ($p = 0.208$) between the occupational groups with (71.3%) and without risk (63.7%). There was also no difference in the analysis on the variable "activities classified as presenting risk" ($p = 0.599$).

Among the inhabitants of the Haliti-Paresí villages studied, the frequency of occurrence of anti-*T. gondii* IgG ranged from 44% to 94.7% (Fig. 1). There were statistically significant differences ($\chi^2 = 37.70$, $p = 0.000$) among the villages.

Chi-square partitions were analyzed by means of calculus to ascertain which villages were directly influencing the result, such that statistical differences were present between them. The villages were evaluated as two different groups, with homogeneity within each group (Table 3).

The villages Bacaiuval/Morrim and Utiariti, which belonged to the blue group, were found to be the drivers of the statistical difference between the villages. The villages in the yellow group did not present significant differences between each other.

Table 1
Frequency of occurrence of anti-*T. gondii* IgG in the Haliti-Paresí indigenous population, according to age group.

Age groups	Tested participants		Seroreactives for IgG anti-*T. gondii*	
	N	%	N	%
0–5	20	6.8	9	45.0
6–10	39	13.3	21	53.9
11–20	64	21.9	44	68.8
21–30	59	20.1	39	66.1
31–40	44	15.0	29	65.9
41–50	28	9.6	20	71.4
+51	39	13.3	34	87.2
Total	293	100	196	66.9

Table 2
Frequency of occurrence of anti-*T. gondii* IgG in the Haliti-Paresí indigenous population according to schooling level.

Schooling	Tested participants		Seroreactives (IgG) anti-*T. gondii*	
	N	%	N	%
Was not in school age	15	5.1	4	26.7
Kindergarten	5	1.7	3	60.0
Incomplete elementary school	143	48.8	97	67.8
Complete elementary school	22	7.5	14	63.6
Incomplete high school	39	13.3	26	66.7
Complete high school	33	11.3	24	72.7
Incomplete higher education	8	2.8	4	50.0
Complete higher education	6	2.0	4	66.7
No year of study	22	7.5	20	90.9
Total	293	100	196	66.9

4. Discussion

The high seroreactivity for IgG that was found in the present study could be correlated with the customs of the indigenous population. Almost all the villagers had the habit of drinking water directly from a river or creek, except in the village Bacaval, where people only drank water from a well. The water for food preparation also came from these rivers, streams and wells.

Based on the above, it can be suggested that the individuals in these villages may have become infected through their water supply, either through directly ingesting water or through consuming food, which in either case may have been contaminated by sporulated oocysts. According to Furtado et al. (2013), consumption of unfiltered water is an important risk factor for *T. gondii* seroreactivity, as also is the washing of vegetables or fruits with untreated water (Ngui et al., 2011).

All of these villages are located near water accumulations, and presence of felids near the courses of the rivers has been reported. This may constitute a risk factor for this population, since these animals may be contributing towards contamination of these waters through sporulated oocysts that are eliminated in their feces. This was previously mentioned by Ngui et al. (2011), who emphasized that felids may come to the river bank or enter the river to drink water or bathe and, at the same time, they may defecate into the water.

The seroreactivity among the population may also be related to these individuals' contact with domestic cats, since domestic cats were present inside the houses in all the villages. Moreover, wild felids were present near the villages, thus enabling contact with and possible ingestion of sporulated oocysts. Tenter et al. (2000) reported that cats were potential transmitters of the protozoon *T. gondii* and Borguezan et al. (2014) added that the presence of cats in houses was one of the main risk factors for zoonoses.

Another risk factor that may have contributed towards high frequency of seroreactivity in the indigenous population was the way in which meat was prepared for consumption. Only in the village Wazare was well-cooked meat consumed; in the other villages, it was consumed raw or minimally cooked. It should be noted that the meat consumed by this population came not only from hunting but also from the market.

The occurrences of anti-*T. gondii* IgM in the present study led us to consider that these results point towards primary infection. However, we cannot consider this to have been recent infection, since some reports in the literature have shown that IgM can be detected for many days, months or even years after the time of the initial infection, remaining at residual levels (Amendoeira, 2001; Liu et al., 2015; Freitas et al., 2017).

The lack of statistical difference in the frequency of occurrence of seropositivity between the sexes confirmed what had been seen in other studies, such as those of Sobral et al. (2005) and Ngui et al. (2011), who affirmed that *Toxoplasma* infection does not distinguish between the sexes, in itself. Individuals' sex could take on some importance if the men and women in a population performed distinct activities that put them at different risks. However, this did not seem to be the case in the present study.

We observed that as age increased, the seroprevalence also increased or was maintained. This demonstrates that the older a person is, the greater the chance of contact with one of the transmission mechanisms also is. These results confirm the findings of Amendoeira et al. (2003).

In the present study, 45% of the children less than five years old were seropositive for anti-*T. gondii* IgG. This suggests that these children had already become exposed to risk factors for acquiring the infection. It is likely that they acquired the infection through their water intake, since children at this age do not consume meat.

The high frequency of seropositivity that was found among women of childbearing age could indicate that congenital transmission of *T. gondii* to their offspring is possible. This seems to confirm the finding of Bóia et al. (2008), who reported the presence of anti-*T. gondii* IgG in women aged 10 to 29 years, and those of Borguezan et al. (2014), who reported the presence of antibodies in indigenous women of childbearing age. The possibility of congenital transmission needs to be borne in mind, given that pregnant women are susceptible to acute *Toxoplasma* infection and that this may increase the risk of such transmission (Lago et al., 2009).

Regarding schooling, the frequency of seropositivity that was found in the samples from Indians who had not attended school at all confirms the finding of Alvarado-Esquivel et al. (2012), who also observed that the highest frequency was among those who had not had any schooling. In the present study, the analysis on the frequency of seropositivity in relation to schooling may also

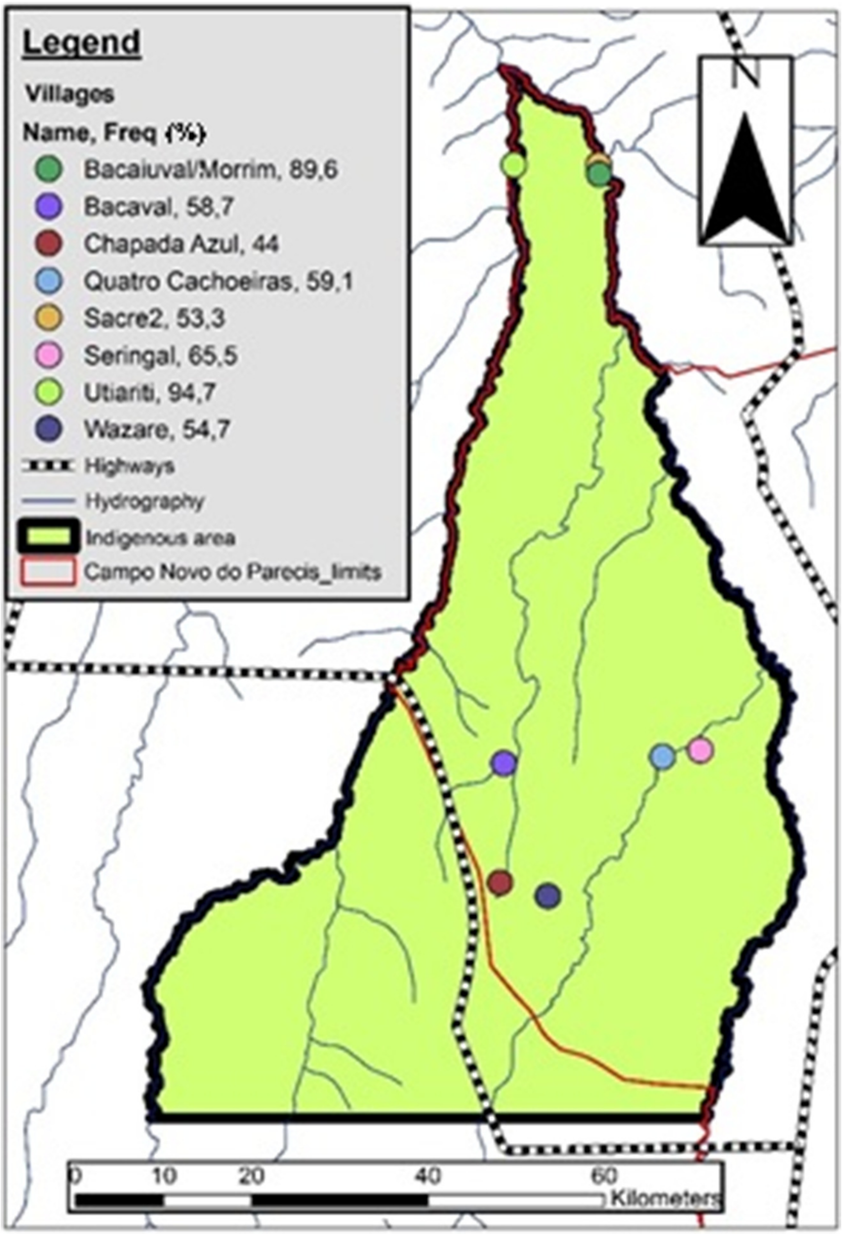

Fig. 1. Frequency of occurrence of anti-*T. gondii* IgG according to the geographical location of each village.
Source: ArcGis 10.0.

have been influenced by age, since younger individuals will have had fewer years of schooling, while the risk of acquiring the infection increases with age.

The variables "occupation/activities with and without risk" and "activities classified as presenting risk" showed that even though different activities were performed by men, women, children, adults and elderly people, all of these individuals came into contact with *T. gondii* and all of them were exposed to the risk of ingesting food and water that were contaminated with sporulated oocysts and tissue cysts containing bradyzoites.

Bacaiuval/Morrim and Utiariti were the villages responsible for the statistical difference in the variable "villages", compared with the other villages. This difference may have been related to the way in which the inhabitants of these villages were ingesting water or food.

The individuals in all the villages in the present study had prevalences of *T. gondii* serogroups that were higher than that found in the indigenous populations of North America, Southeast Asia, and East Asia. These prevalences ranged from 10.6% to 40.6% (Hakim et al., 1994; Ngui et al., 2011; Alvarado-Esquivel et al., 2012; Lin et al., 2008; Alvarado-Esquivel et al., 2016).

Table 3
Chi-square partitions between the Haliti-Paresí indigenous villages.

Villages	ELISA		Total	Pro[1]	ProA[2]	∑ProA[3]	Groups
	Non-seroreactive	Seroreactive					
Chapada Azul	14	11	25	0,440	4,840	139,461	
Sacre 2	7	8	15	0,533	4,267		
Wazare	24	29	53	0,547	15,868		
Bacaval	26	37	63	0,587	21,730		
Quatro Cachoeiras	9	13	22	0,591	7,682		
Seringal/ Cabeceira do Seringal	10	19	29	0,655	12,448		
Bacaiuval/ Morrim	5	43	48	0,896	38,521		
Utiariti	2	36	38	0,947	34,105		
Total	**97**	**196**	**293**	**0,669**	**131,113**		

[1]Division of seroreagents by total.
[2]Multiplication of "Pro" with seroreagents.
[3]Sum of "ProA".

The frequencies of seroreactivity for *T. gondii* among the inhabitants of the villages Utiariti and Bacaiuval/Morrim were higher than those seen in the study by Amendoeira et al. (2003) (80.4%). On the other hand, the findings from the villages Seringal/Cabeceira do Seringal, Quatro Cachoeiras and Bacaval were similar to those of Díaz-Suárez et al. (2003), who found seroreactivity of 62.7%; Bóia et al. (2008), 73.5% and Devera et al. (2013), 51.63%.

In the villages Wazare and Sacre 2, the frequencies of seropositivity were similar to those of Baruzzi (1970), who found a rate of 51.6%, and Sobral et al. (2005) in the villages Waiãpi (59.6%) and Tiriyó (55.6%). The rate in the village Chapada Azul was similar to those of the Barí Amerindians of the communities Saimadoyi (49.6%), Karakñakayek (41.8%) and Bakugbarí (38.2%) in Venezuela (Chacin-Bonilla et al., 2001).

5. Conclusion

In the present study, high frequency of anti-*Toxoplasma gondii* antibodies of the IgG class was found in the Haliti-Paresí indigenous population. Moreover, 3.4% of the population were found to be seroreactive for IgM. The findings of seroreactivity to IgG among children younger than five years of age suggested that transmission was occurring in the region. This emphasizes the importance of surveillance of transmission of infection caused by this parasite, especially in relation to the 30% of the women of fertile age (from 14 to 40 years) who were seronegative and would be at risk of acquiring the infection during pregnancy.

Declaration of interest

The authors report no competing interests exist.

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