

SEROPREVALENCE OF INFECTION WITH *TOXOPLASMA GONDII* IN INDIGENOUS BRAZILIAN POPULATIONS

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Abstract. The prevalence of *Toxoplasma gondii* in indigenous Brazilian tribes with different degrees of acculturation was studied in the Enawenê-Nawê, an isolated tribe, in the state of Mato Grosso, the Waiãpi, with intermittent non-Indian contacts, in the state of Amapá, and the Tiriýó, with constant non-Indian contacts, in the state of Pará. An IgG–enzyme-linked immunosorbent assay (IgG-ELISA) or an IgG/IgM–indirect immunofluorescence antibody (IFA) assay were performed for the detection of antibodies to *T. gondii* in 2000–2001. Both assays showed that the Tiriýó had the lowest crude seroprevalence (55.6%), the Enawenê-Nawê the highest crude seroprevalence (80.4%), and the Waiãpi an intermediate crude seroprevalence (59.6%). The age-adjusted prevalence (95% confidence intervals) values for the Tiriýó, Enawenê-Nawê, and Waiãpi were 57.3% (53.4, 61.1%), 78.8% (72.2, 85.7%), and 57.7% (52.5, 62.9%), respectively. Contact with non-Indians probably did not influence the prevalence of the infection. However, differential contact with soil-harboring oocysts from wild felines may be responsible for the various seroprevalences in the different tribes.

INTRODUCTION

Toxoplasmosis is a zoonosis of worldwide distribution that is present in many diverse areas, such as hot, humid regions in the Amazon Basin¹ and cold regions in the Arctic and Alaska.² Previous studies of the prevalence of infection with *Toxoplasma gondii* in Brazilian Indians have shown a variation in the prevalence between 39% and 100%.^{3–6} Among the regions inhabited by the primitive tribes of New Guinea, where the distribution of domestic cats and wild felines in these regions was limited or nonexistent, Wallace and others⁷ showed that the prevalence of antibodies to *T. gondii* was 2%. Using the Sabin Feldman test, they also showed that the prevalence of antibodies to *T. gondii* in this population ranged between 14% and 30% in areas where numerous cats were present.⁷

The study of indigenous populations is useful in understanding the relationship between the source of infection with *T. gondii* and its occurrence, especially since these communities usually have long-established customs and habits. Furthermore, the study of their lifestyles may facilitate the identification of the source of the infection and show the patterns of transmission.

The objective of this study was to determine the prevalence of infection with *T. gondii* among three indigenous populations in Brazil with different degrees of acculturation (isolated, intermittent contact, and continuous contact with other groups) and the presence of different environmental risk factors. Seroprevalence was determined with respect to tribal ethnicity, geographic region, and sex.

POPULATIONS AND METHODS

Characteristics of the study populations. *Isolated population: Enawenê-Nawê.* The Enawenê-Nawê live in an indigenous area of 750,000 hectares in the northwestern area of the State of the Mato Grosso in Brazil (Figure 1). In 1995, a population census showed that there were 245 individuals; the most recent information is that there are now 320 individuals.⁸ This population cultivates corn, cassava, leguminous plants, and fish. They do not consume any red meat, but do eat insects. Honey is a product of great importance in their

diet and it is collected only by the men. Mushrooms found on the bank of rivers are also part of the diet. The women and children collect the mushrooms, which are consumed raw before or after washing in water. The Indians maintain frequent contact with the soil and water of the rivers and do not breed any domestic animals, but there are wild animals, including felines, in the vicinity of the villages, and at the sites of water collection. This population is isolated without any direct contact with non-Indians, except for the contacts with the physicians and staff of the medical team of the Brazilian National Agency for Indian Affairs. The village has a circular configuration, with 10 rectangular communal houses distributed radially, and a single house located in the center.⁹

Intermittent contact population: Waiãpi. The Waiãpi, who belong to the Tupi-Guarani language group, inhabit a vast area of forest along the border between Brazil and Guyana (Figure 1). The total population of this community is approximately 1,200 people distributed in several territorial groups in Brazil, the current population is concentrated in state of Amapá. The population in Brazilian consists of approximately 450 people distributed in 11 villages and fixed camps dispersed over an area of 543,000 hectares.¹⁰ The territory of the Waiãpi Indians is tropical forest. The principal characteristic of the Waiãpi is their constant mobility among several areas in their territory, where they hunt and fish. The Waiãpi complete their subsistence diet with the collection of several products such as regional fruits, honey, and adult insects, including their larva.¹¹ Housing is extremely diversified and does not follow any rigid pattern. Some villages have only one or two houses, whereas other villages have more than 15 houses. Agriculture is a central activity in the life of the Waiãpi. They also mine gold on a small scale, and this activity is integrated into the cycle of traditional activities.¹¹

Constant contact population: Tiriýó. The Tiriýó inhabit the frontier area along the border between northern Brazil and Suriname. They are composed of a population of 1,700 individuals, of whom which 750 live in Brazil, and inhabit an area in the northwestern part of the Tumucumaque Park. The region is characterized geographically by the plains, fields, forests and mountains in northern Pará (Figure 1). Beginning in 1960, they began to settle with missionaries. They are currently distributed in Brazil among 12 villages close to the base

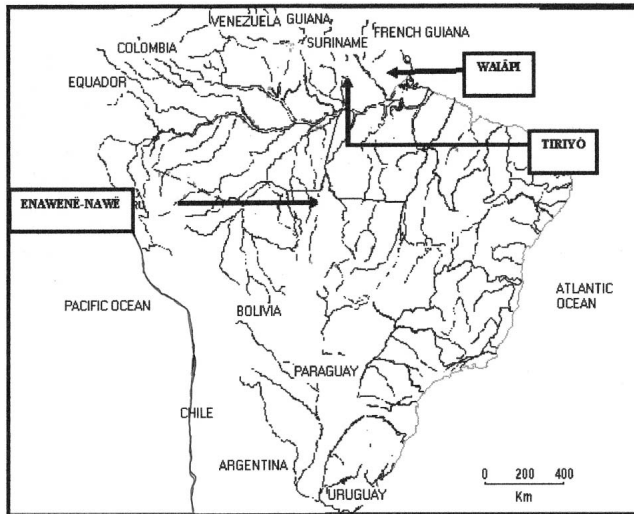


FIGURE 1. Map of Brazil showing the locations of the three tribes studied.

of the Franciscan Mission known as “Mission Tiriós.”¹⁰ The “Mission Tiriós” has an infrastructure with several improvements, such as chlorinated water and electricity, and resembles any small city in interior Brazil.

Ethical guidelines. Research projects dealing with the health of the indigenous Indian tribes in the eastern, central, and northern Brazil are authorized after vigorous examination by several authorities in Brazil. The Operação Amazônia Nativa (Native Amazonia Operation) reviewed and approved the current project. In addition, this project was reviewed and approved by the ethical commission of the Oswaldo Cruz Foundation (Project 25000.098034/2001-46) and the National Commission on Ethics in Research. Informed consent was obtained from all the members of the Indian tribes and their children through help from the Brazilian National Agency for Indian Affairs/Brazilian National Health Foundation of the Ministry of Health. This study is an integral part of the project of “Health of the Indigenous People of Brazil.”

Serologic study. All blood samples were collected by vein puncture. Blood samples were collected from 148 individuals in the Enawenê-Nawê tribe. This sample represented 60.4% of the total population ($n = 245$) and included men, women, and children 6–75 years of age. Similarly, 302 blood samples were collected from individuals 4–69 years old of both sexes in the Waiãpi tribe. This represented 67.1% of the total population ($n = 450$). Five hundred sixty-eight blood samples were collected from individuals 4–90 years old of both sexes in the Tiriyo tribe. This represented 75.7% of the total population ($n = 750$).

Laboratory methods. Analysis of serum samples from 1,018 individuals of the three indigenous tribes was carried out at the FIOCRUZ Toxoplasmosis Laboratory in Rio de Janeiro. An enzyme-linked immunosorbent assay (ELISA) was used for detection of IgG antibodies to *T. gondii*.¹² The cut-off point for the IgG-ELISA was established by assaying 12 negative standard serum samples and four positive serum samples on four different plates. The cut-off for each plate consisted of the mean reading of the negative serum sample plus two standard deviations. A correction factor was determined by dividing the median of the cut-off value by the average reading of the negative sera in these plates.

An indirect immunofluorescence antibody (IFA) assay was used for the detection of IgM antibodies and specific IgG antibodies. Four different dilutions of serum ranging from 1:16 to 1:4,096 were made in phosphate-buffered saline. For detection, conjugated anti-IgM and anti-IgG human fluorescent antibodies diluted 1:50 were used. This dilution was established after analysis with standard reactive and non-reactive serum. A reaction with a serum dilution $\geq 1:16$ was considered reactive, and the final titer was the last dilution that still showed fluorescence in the periphery of the parasites. The fluorescent tests were performed according to the method of Camargo.¹³ The material was examined using an epi-fluorescence microscope (Y-FL; Nikon, Tokyo, Japan) using a 40 \times objective, a 10 \times ocular lens, an ND16 exciting filter, and a mercury lamp.

The IFA assay was standardized in our laboratory and showed comparable sensitivity and specificity as that of the Sabin Feldman dye test, which is regarded as the gold standard. The titers measured by the IFA assay and the dye test are comparable.^{13,14} All serum samples in the IgM-IFA assay were tested for the presence of the rheumatoid antibodies using a diagnostic kit (Bio Lab Mérieux SA, Rio de Janeiro, Brazil).¹⁵ Serum reactive for *T. gondii* were retested with an immunoenzymatic assay for IgM antibodies (Platelia® *Toxoplasma gondii* IgM tetramethylbenzidine; Bio-Rad Laboratories, Marnes la Coquette, France).

Statistical analysis. Chi-square tests were used to compare the prevalence of seropositivity in relation to the sex and age. A P value ≤ 0.05 was considered significant.¹⁶ A non-parametric test of trend in ordered groups was also used.¹⁷ The prevalence of infection among the indigenous populations was standardized by the adjusting direct method.¹⁸ Finally, to locate heterogeneity in the distributions of the antibody titers, partition of chi-square was performed.¹⁶ The Kappa statistic was determined and comparison between the two diagnostic tests against the same gold standard in the same sample (IFA assay as the gold standard) was performed.^{19,20}

RESULTS

The results of the ELISA and IFA assay for detecting the antibodies against *T. gondii* showed positive serology in 80.4% in the Enawenê-Nawê, 59.6% in the Waiãpi, and 55.6% in the Tiriyo. None of the Enawenê-Nawê tested positive in the IgM-IFA assay, but five individuals in the Waiãpi and one individual in the Tiriyo tested positive.

The samples was stratified and analyzed by sex to evaluate its effect on the distribution of infection with *T. gondii*. In the Enawenê-Nawê, 86.1% of the males and 75.9% of the females were positive for IgG antibodies ($\chi^2 = 2.43$, $P = 0.119$). In the Waiãpi 59.2% of the males and 60.0% of the females were positive ($\chi^2 = 0.0183$, $P = 0.892$). In the Tiriyo, 59.2% of the males and 52.1% of the females were positive ($\chi^2 = 2.92$, $P = 0.088$).

Significant heterogeneity was observed among the age groups in the three tribes studied. A non-parametric test showed a significant trend of antibody positivity increasing with age (Table 1).

To compare the prevalence of seropositivity in the three tribes, the percentages of those who were reactive were ad-

TABLE 1
Seroprevalence of IgG antibody to *Toxoplasma gondii* (by ELISA or IFA) in the three indigenous groups according to age*

Group	Age (years)						Total
	4-9	10-19	20-29	30-39	40-49	≥50	
Enawenê-Nawê	50.0% (12)	71.7% (53)	85.1% (27)	92.3% (26)	90.0% (10)	95.0% (20)	80.4% (148)
Waiãpi	25.0% (20)	54.9% (113)	55.0% (80)	69.8% (43)	80.9% (21)	88.0% (25)	59.6% (302)
Tiriyó	27.4% (113)	49.4% (174)	57.6% (111)	70.7% (75)	84.4% (32)	87.3% (63)	55.6% (568)
Total	14.2% (145)	33.4% (340)	21.4% (218)	14.1% (144)	6.19% (63)	10.6% (108)	60.4% (1,018)

* Values in parentheses are the total number of Indians tested in each group. ELISA = enzyme-linked immunosorbent assay; IFA = indirect immunofluorescence antibody (assay). Enawenê-Nawê: $\chi^2 = 15.61$, $P < 0.01$, $Z = 3.49$, $P < 0.01$; Waiãpi: $\chi^2 = 25.90$; $P < 0.01$, $Z = 4.69$, $P < 0.01$; Tiriyó: $\chi^2 = 82.48$; $P < 0.01$, $Z = 8.86$, $P < 0.01$.

justed by the direct method of standardization of coefficients (population pattern is composed of the three tribes). This arrangement showed that the Enawenê-Nawê had an adjusted estimate that was significantly different from those of the other tribes studied. As seen in Table 2, the point estimates showed that the percentage of IgG reactivity in the Enawenê-Nawê exceeded that of the other two groups. Significant differences were observed in the adjusted IgG reactive prevalence of the Enawenê-Nawê (78.8%, 95% confidence interval [CI] = 72.2, 85.7%) compared with the Waiãpi (57.7%, 95% CI = 52.5, 62.9%) and the Tiriyó (57.3%, 95% CI = 53.4, 61.1%). The distribution of IgG titers determined by the IFA assay is shown in Table 3. In the Enawenê-Nawê, for most dilutions the percentage of reactive sera was higher than for the other two groups. Since the homogeneity of the distribution of reactive sera was rejected ($P < 0.0001$, by chi-square test) (Table 3), a partition was performed to locate the heterogeneity. As shown in Table 4, the differences in the distributions of the titers were clearer when the Enawenê-Nawê was compared with the combined results of the other two tribes.

The distribution of IgG titers determined by the IFA assay and the ELISA is shown in Table 5. A comparison of these two diagnostic tests using the IFA assay as the gold standard showed kappa = 0.57 (95% CI = 0.52, 0.62), a sensitivity (copositivity) of 84% (95% CI = 0.73, 82%), and a specificity (conegativity) of 74% (95% CI = 0.70, 0.77%).

DISCUSSION

The highest prevalence (80.4%) of antibodies to *T. gondii* was detected in the Enawenê-Nawê. This level of antibodies

TABLE 2

Percentage of IgG crude and adjusted* reactivity (ELISA or IFA) in the indigenous groups†

Indigenous group	% crude IgG	% adjusted IgG	95% confidence interval of the % adjusted IgG	Number of individuals
Enawenê-Nawê	80.4	78.8	(72.2, 85.7)	148
Waiãpi	59.6	57.7	(52.5, 62.9)	302
Tiriyó	55.6	57.3	(53.4, 61.1)	568

* Direct method of standardization of coefficients.

† The confidence intervals were calculated after the estimation of standard errors in stratified sampling for proportions, with weights for strata derived from the standard population (sum of the three groups²³). ELISA = enzyme-linked immunosorbent assay; IFA = indirect immunofluorescence antibody (assay).

to *T. gondii* is similar to that found in other Brazilian Indian tribes with similar characteristics, such as the Kren-Akore, who showed a seropositivity of 88.6%. The Kren-Akore Indians had their first contact with non-Indians two years before the commencement of this study.²¹ Among the Amerindians of the Guajiba tribe of the Amazon jungle in Venezuela, a seropositivity of 88% was reported.²²

The high prevalence of antibodies to *T. gondii* in the Enawenê-Nawê cannot be easily explained by the more common forms of transmission of toxoplasmosis, such as the ingestion of cysts present in raw meat or inadequately cooked meat and the presence of oocysts in the feces of cats. This is because the Enawenê-Nawê eat fish, but do not consume any red meat and do not have cats as pets. Furthermore, the low density of wild felines dispersed over large areas does not result in the high levels of soil contamination that explain the high levels of antibodies to *T. gondii* in the Enawenê-Nawê. A possible source could be the concentration of wild felines at water collection sites used by these Indians. At these sites, as in other areas of Brazil, unfiltered water has been considered a risk factor for infection with *T. gondii*.²³ The living habits of the Enawenê-Nawê and Waiãpi are similar in relation to their contacts with the soil and the consumption of larva and insects. Therefore, this can expose them to the oocysts of *T. gondii*. It was expected that the prevalence of the infection among these two tribes would be similar and different from that of the Tiriyó, whose living habits are closer to those of the non-Indians. However, the seroprevalence of *T. gondii* among the Waiãpi and the Tiriyó were very similar, 59.6%

TABLE 3

Frequency (%) of IgG antibody titers to *Toxoplasma gondii* determined by IFA in the three indigenous groups*

IFA titers	Indigenous tribes			
	Enawenê-Nawê	Waiãpi	Tiriyó	Total
Non-reactive	27.7	55.6	59.3	53.6
1:16	15.5	21.8	21.1	20.5
1:64	29.1	13.9	10.4	14.2
1:256	19.6	5.3	6.3	8.0
1:1,024	7.4	2.6	2.6	3.3
1:4,096	0.68	0.66	0.18	0.39
Total	100 (148)	100 (302)	100 (568)	100 (1,018)

* Values in parentheses are the total number of Indians tested in each group. IFA = indirect immunofluorescence antibody (assay). $\chi^2 = 93.2751$, degrees of freedom = 10, $P < 0.01$.

TABLE 4

Tests of homogeneity of the distributions of IgG titers to *Toxoplasma gondii* determined by IFA in the three indigenous groups*

	χ^2	DF	P
Enawenê-Nawê × Waiãpi × Tiriyó	93.28	10	< 0.01
Tiriyó × Waiãpi	4.33	5	0.50
Enawenê-Nawê × (Tiriyó + Waiãpi)	89.54	5	< 0.01

* IFA = indirect immunofluorescence antibody (assay); DF = degrees of freedom.

and 55.6% respectively, and different from that of the Enawenê-Nawê.

Some investigators attribute great importance to the consumption of red meat and its relationship to the high seropositivity of antibodies to *T. gondii*.⁶ This fact is supported by a study performed with the Ticuna Indians, in whom antibody titers $\geq 1:256$ were higher in some villages, where the alimentary habits were more varied, and similar to populations of non-Indians, than in the villages whose source of animal protein was predominantly fish.⁶ However in our study of the Enawenê-Nawê, who do not consume any red meat, the frequency of antibody titers $\geq 1:256$ in 27.7% was higher than among the populations of Tiriyó (9.08%) and Waiãpi (8.56%), whose diets include this type of food.

No significant differences with respect to infection with *T. gondii* were detected between males and the females in the three tribes studied. These findings are similar to those described in the Indians of High Xingu, among whom 46.9% of the men and 56.5% of the women tested positive for antibodies to *T. gondii*.⁴ A similar study in the Amapá showed that 71.2% the males and 66.5% of the females were positive for antibodies to *T. gondii*.²⁴ These results suggest that the rate of infection for *T. gondii* is not sex related, or that the differences observed are not important, bearing in mind the fact that activities performed by men and women among these three tribes are quite different and that most of them had varying degrees of frequent contact with risk factors.

The high frequency of low IFA assay titers of 1:16 and 1:64 to *T. gondii* observed among the three indigenous tribes studied (Table 3) are similar to those detected among the natives of High Xingu (51.1%), in whom IFA assay titers varied from 1:16 to 1:256.⁴ Among the populations studied, only four males had titers of 1:4,096: one in the Enawenê-Nawê, two in the Waiãpi, and one in the Tiriyó. None of the Enawenê-Nawê showed reactivity for IgM. Five of the Waiãpi and one of the Tiriyó were reactive for IgM. With a smaller number of IgM-reactive individuals and a larger number of IgG-reactive individuals among these tribes, there may be chronic infection with *T. gondii* among these three tribes. This was also sug-

TABLE 5

Serologic results of tests for antibodies to *Toxoplasma gondii* in the three indigenous groups*

IFA	ELISA		Total
	Reactive	Non-reactive	
Reactive	396	76	472
Non-reactive	143	403	546
Total	539	479	1,018

* ELISA = enzyme-linked immunosorbent assay; IFA = indirect immunofluorescence antibody (assay), kappa = 0.57 (95% CI = 0.52-0.62); sensitivity (cpositivity) = 84% (95% CI = 0.73-0.82%); specificity (conegativity) = 74% (95% CI = 0.70-0.77%).

gested by Coimbra and Santos,²⁵ who indicated that toxoplasmosis was an endemic zoonosis."

With regard to the age groups seropositivity in the Enawenê-Nawê begins to plateau starting from the third decade of life. However, this occurs later in the other two tribes. As in other studies,^{26,27} we found that the frequency of infection with *T. gondii* increases with age. This is because with age, the probability that an individual may make contact with at least one of the transmission mechanisms also increases.^{28,29}

The high frequency of infection with *T. gondii* in younger age groups suggests that these children have already been exposed to risk factors for acquiring the infection because 50% of the children less than 10 years old were seropositive by the ELISA or IFA assay and four of them had IgG-IFA assay antibodies titers > 1:64.

The harmful effects of the acculturation process in the health of the Amerindians are well documented in relation to the presence of many infections such as hepatitis, tuberculosis, and other viral diseases. In a study of the Amerindians of Venezuela, it was observed that the acculturation process elevated the prevalence of toxoplasmosis because those individuals had started to have a more sedentary lifestyle, including the presence of domestic cats in households and the use of a community water supply.³⁰

However, in group studies, the degree of contact with non-Indians does not seem to be responsible for an increase in the seropositivity, which seems to be more related to the life and cultural habits of each of the Indian populations. The Tiriyó showed an infection rate for *T. gondii* of 43% in 1974 (150 analyzed by the dye test) when they were considered an isolated population.³¹ Currently, the Tiriyó have much greater contact with the non-Indians, a higher degree of acculturation, and the infection rate for *T. gondii* has increased to 55.6% (568 tested). This is a significant increase when compared with the previous rate ($\chi^2 = 7.50$, $P = 0.006$). In the Tiriyó, contact with non-Indians did not influence the activities of the group that could expose them to risk factors for infection with *T. gondii*. In the present study, the Enawenê-Nawê, who had fewer contacts with non-Indians, showed the highest seroprevalence for toxoplasmosis.

When we analyzed the three indigenous populations and adjusted for confounding effects caused by sex and age, for the direct method of standardization of the coefficients (Table 2), it was observed that the rates of seropositivity in the Waiãpi and of the Tiriyó were similar. Furthermore, it was observed that the seropositivity and the distribution of the IFA assay titers in the Enawenê-Nawê were different from those of the other tribes. Therefore, it is suggested that the Enawenê-Nawê are in contact with some other risk factor(s) that lead to an increase in the prevalence of antibodies to *T. gondii*, and that these factors are not as common in the other two tribes studied.

The Enawenê-Nawê have different dietary habits compared with the Waiãpi and the Tiriyó, mainly with respect to the consumption of mushrooms located in the forests with large amounts of organic matter in the soil. In addition, the living area of the Enawenê-Nawê may be visited by wild felines in search of water and could become contaminated with oocysts eliminated in their feces. Thus, exposure to this environment may result in the difference in seropositivity for *T. gondii* in the Enawenê-Nawê in comparison to the other two tribes studied.

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