Seroprevalence of toxoplasmosis, toxocariasis and cysticercosis in a rural settlement, São Paulo State, Brazil

Luiz Euribel Prestes-Carneiro¹, Guita Rubinsky-Elefant², Antonio Walter Ferreira³, Patricia Regina Araujo³, Charlene Troiani¹, Sueli Cristina Zago¹, Marcia Kaiahara¹, Leticia Sasso⁴, Alberto Iha⁵, Adelaide Vaz 'In memoriam'⁵

¹Universidade do Oeste Paulista, Department of Immunology, Presidente Prudente, SP, Brazil, ²Institute of Tropical Medicine of São Paulo, Laboratory of Seroepidemiology and Immunobiology, University of São Paulo, São Paulo, SP, Brazil, ³Department of Infectious and Parasitic Diseases, São Paulo, SP, Brazil, ⁴Department of Ophthalmology, University of Oeste Paulista, Presidente Prudente, SP, Brazil, ⁵Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil

Background: The goal of this study was to estimate the seroprevalence of *Toxocara* spp., *Toxoplasma gondii*, and *Taenia solium metacestode* infection and determine some of the associated risk factors for people living in the Dona Carmen settlement, Pontal of Paranapanema, São Paulo, Brazil.

Methods: Serum samples from 194 subjects were tested and participants answered a questionnaire. An enzyme-linked immunosorbent assay (ELISA) system based on *Toxocara* spp. excretory-secretory antigens obtained from the cultured second-stage larvae of *Toxocara canis* or vesicular fluid (VF) antigen from *Taenia crassiceps metacestode* was used to detect anti-*Toxocara* spp. IgG and IgE and anti-*T. solium metacestode*, respectively. For cysticercosis, the reactive ELISA samples were assayed by Western blotting using 18 kDa and 14 kDa proteins purified from VF. For *T. gondii*-specific IgG and IgM antibodies, anti-SAG-1, GRA-1, and GRA-7 epitope specificity was determined by ELISA.

Results: *Toxoplasma gondii* IgG antibodies were found in 102/194 individuals (52.6%) with increased infections in females ($P = 0.02$) and those with $\leq$ US\$300 monthly income ($P = 0.01$). Positive IgM antibodies were detected in 21/194 individuals (10.8%). Antibodies specific to *Toxocara* spp. were found in 28/194 subjects (14.4%). All the individuals with *Toxocara* spp. also had *T. gondii*-specific IgG antibodies. *Taenia solium metacestode* antibodies were detected in 11 subjects (5.7%), but none were reactive based on Western blotting.

Conclusion: In spite of environmental, educational, and socioeconomic factors favoring parasite infection, the seropositivity rates of *T. gondii*, *Toxocara* spp., and *T. solium metacestode*-specific IgG antibodies are similar to the rates found in studies conducted in different populations in Brazil.

Keywords: *Toxocara* spp., Toxoplasmosis, Cysticercosis, Seroprevalence, Rural settlement, Brazil

Introduction

Human toxocariasis is caused by larval stages of both *Toxocara canis* and *Toxocara cati*, which results from ingesting embryonated eggs present mainly in contaminated soil or sand; toxoplasmosis is caused by the intracellular protozoa *Toxoplasma gondii* and results from ingesting infective oocysts, and is also transmitted by bloodstream or congenital acquired infection. These are zoonoses whose main reservoirs are both dogs and cats.¹ These diseases are some of the most prevalent zoonoses worldwide due to increasing numbers of pets living together with

humans.^{1,2} On the other hand, cysticercosis is caused by larval stages of *Taenia solium* and results from ingesting eggs released with feces from parasitized individuals with the adult tapeworm.³ These pathogens are important public health problems that especially affect people living in rural areas with poor sanitation and low economic and educational levels.¹⁻³

After invading the host, these pathogens use common routes to reach their target tissues. *Toxocara* spp. and *T. gondii* can target the liver or the eye, leading to ocular damage.^{1,2} *T. solium* can invade the central nervous system and lead to neurocysticercosis.^{3,4} These pathogens have also been linked to neuronal manifestations.³⁻⁵

In Brazil, seroprevalence of these agents varies according to geographical region, biological characteristics, and

Correspondence to: Luiz Euribel Prestes-Carneiro, Universidade do Oeste Paulista, Rua José Bongiovani 700, Cidade Universitária, Presidente Prudente, São Paulo, Brazil. Email: luiz@unoeste.br

the local environment.⁶⁻⁸ The diagnosis of toxocariasis, toxoplasmosis, and cysticercosis are based on clinical examination, diagnostic images, and serology.⁹⁻¹¹ For the serodiagnosis of toxocariasis, an enzyme-linked immunosorbent assay (ELISA) using *T. canis* larvae excretory-secretory antigens (TES) is widely used to detect specific IgG antibodies.⁹ For acute toxoplasmosis, the serodiagnosis is made by detecting specific IgM antibodies and IgG through an avidity test.¹¹ The assay of choice for serodiagnosis of cysticercosis in humans is the immunoblot assay using the lentil lectin chromatographically purified (CP) glycoproteins antigens derived from *T. solium metacetodes*.¹² However, other working groups have found that antigens from other species may be useful for diagnosing cysticercosis, such as 14 and 18 kDa purified antigens from *Taenia crassiceps*.^{10,13} In poor resourcing settings, an ELISA test may be applied for screening the disease. However, for all of these agents, false-negative and false-positive ELISA results have been observed.⁹⁻¹¹

Invasion and formation of a settlement beside the area to be occupied is one of the first steps used by landless worker movements in Brazil to pressurize the local authorities against expropriation, distribution, and settlement arrangements. Dozens of new settlements have been formed in the past decades in the Pontal of Paranapanema region, and there are disputes over land between landless workers and cattle farmers.¹⁴ Throughout the country, the families in these settlements live in plastic huts with marginal roads, sometimes for periods as long as 2–4 years. These settlements are characterized by poor living conditions, deficient potable water supplies and sanitary facilities and could be a source for parasitic infections.^{14,15}

The Pontal of Paranapanema is one of the poorest and most undeveloped regions of the São Paulo state, in southeastern Brazil. This region contains the highest density of rural settlements in the country.¹⁴⁻¹⁶ We have previously determined the seroprevalence of toxocariasis/cysticercosis in this region in both an old settlement (20 years) and in a recently formed settlement (2 years).¹⁴⁻¹⁶ The aim of this study was to determine the seroprevalence of *Toxocara* spp., *T. gondii*, and *T. solium metacestode* infection and explore some risk factors in people living in a settlement in Pontal of Paranapanema, São Paulo, Brazil.

Materials and Methods

Study design

The Dona Carmen settlement is located in Mirante of Paranapanema County, Brazil and has been organized by the Landless Workers Movement (MST) since 2006, harboring approximately 100 families with 560 individuals. Most of these families live in shacks built from scrap wood and canvas with asbestos tile roofs, without spatial distribution, and

beside a district freeway (Fig. 1). Each family receives a box with basic food from the Federal Government monthly. Medical assistance and health educational program are provided by multi-disciplinary family health professionals (ESF).

The present study is a transversal and descriptive study, and all specimens were collected from December 2008 to December 2009 from the 194 individuals who agreed to take part in this study, representing 34.64% of the 560 inhabitants living in the settlement. The study participants were randomly selected from healthy volunteers, and ranged in age from 5 to 73 years. Informed consent was obtained from all of the included subjects prior to their participation in the investigation. In order to obtain information on risks factors, a short questionnaire interview was conducted, which included age (classified as <13 years and ≥13 years), gender, place of birth, educational level, pregnancy, source of water supply, income, sanitary facilities, and whether their housing consisted of plastic, wood, or brick. We also assessed whether the subjects had clinical manifestations associated with the studied zoonoses (nausea, vomiting, headache, epilepsy, convulsion, seizures, and anxiety) and whether they were involved in raising cattle, pigs, dogs or cats.

Hematological tests

Hemogram was carried out using a flow cytometer flux counter, and the differential leukocyte count was checked and compared with the direct microscopic observation of blood smears. The level of eosinophilia was assessed as the percentage of leucocytes represented by eosinophils.¹⁷

Total IgE

Total IgE was measured in serum samples using an automated chemiluminescence assay (Immulite Diagnostic Products Corporation, Los Angeles, CA, USA).

Toxocara canis-specific ELISA

Specific anti-*T. canis* IgG and IgE antibodies were detected by ELISA using TES antigens based on the method described by De Savigny *et al.*,¹⁸ with some modifications of Elefant *et al.*⁹ The test is unable to distinguish between exposures to *T. canis* and *T. cati*. Non-specific antibodies were removed by serum absorption with *Ascaris lumbricoides* adult worm extracts.^{10,19}

Taenia crassiceps metacestode ELISA (ELISA-VF)

Anti-*T. solium metacestode* antibodies were detected by ELISA as described by Espíndola *et al.*¹³ with some modifications. The antigens consisted of 14–18 kDa proteins from the vesicular fluid (VF) of *T. crassiceps cysticerci* (Tcra).^{10,13}

Figure 1 Overview of Dona Carmen settlement located in Mirante of Paranapanema County, São Paulo State, Brazil.

Taenia crassiceps metacestode Immunoblot (IB-18/14)

All the serum samples that were reactive in the ELISA screening were confirmed by immunoblotting using 18/14-Tcra proteins to measure antibody specificity. The 18/14-Tcra antigens were obtained by affinity chromatography with specific monoclonal antibodies, using a previously described methodology.^{14,16}

Toxoplasma gondii IgG and IgM

Toxoplasma gondii IgG and IgM antibodies were detected using ELISA containing *T. gondii* excretory-secretory antigen (ESA) peptides.^{11,20} The ESA peptides chosen were granule protein 1 (GRA-1), which correlates with chronic toxoplasmosis, granule protein 7 (GRA-7) which is detected in acute toxoplasmosis,^{21,22} and surface antigen 1 (SAG-1), which is present only in the tachyzoite stage and is a marker of acute toxoplasmosis,²³ based on the methodology described by Araújo and Ferreira.^{11,20}

Ophthalmologic examination

Patients who were seropositive to specific IgG for *Toxocara* spp. and specific IgM for *T. gondii* were examined by a physician and then by an ophthalmologist using an indirect funduscopy ocular examination following mydriatic treatment with tropicamide and fenilefrine.

Coproparasitologic studies

Stools were collected and examined using Faust and Hoffmann and Pons and Janner methods, for the detection of cysts, parasites, or adult proglottids.¹⁴

Data analysis

Independent sample *t*-tests analyses were used to compare *Toxocara* IgG antibody levels with the presence of dogs, family income, or the subject's education level. When indicated, chi-square (χ^2), Fisher exact, and the correlation tests were applied using GraphPad software (V4.0) (San Diego, CA, USA) and Sigma-Stat software (Systat Software Inc., Richmond, CA, USA). The statistical significance

(5%) of differences among groups was assessed by testing the regression coefficients.

Results

Population characteristics

A total of 194 individuals (34.6%) living in the Dona Carmen settlement were analyzed. Among them, 101 (52.0%) were male and 93 (48.0%) were female. The male:female ratio was 1:1, with ages ranging from 5 to 73 years old and a mean age of 32.2 (29.76–34.85; CI 95%) years. A total of 38 (19.6%) individuals were less than 13 years old. Before occupying the settlement, 123 (63.4%) individuals lived in the São Paulo State, 64 (33.0%) lived in the neighboring state of Paraná, and 7 (3.6%) in the northeast states of Brazil. A total of 117 (60.3%) had already lived in other settlements before coming to Dona Carmen. The average time of living in the other places was 3.33 ± 4.48 years (CI 2.53–4.13). The main symptoms found in the studied population were: headache (33%), skin allergy (24.7%), vertigo (23.2%), and bilateral visual symptoms (18.6%). With regard to risks factors, 12.9% had onychophagy and 3.1% had geophagy; 58.2% of them reported eating undercooked vegetables and 18.6% reported eating raw pork or cattle meat. The source of water varied among the studied subjects: 160 (82.5%) of them used ground floor wells; 31 (16.0%) of them used a communitarian artesian well and three (1.5%) of them just used a small and a big river beside the settlement (Fig. 1). No statistical significance was found among individuals who used either artesian or ground floor wells or rivers as their main source for water and getting infected with toxocariasis ($P=1.0$) or toxoplasmosis ($P = 0.77$). Sanitary facilities were also assessed: 77.8% had access to a sewer and 5.1% had access to a cesspool but 17% of them had access to neither a cesspool nor a sewer.

Seroprevalence of *T. gondii*-specific IgM and IgG antibodies and associated risk factors

Anti-*T. gondii* IgG antibodies were found in 102/194 (52.6%) individuals. Among the associated potential risk factors for seropositivity, a significant increase in infection was found in females ($P=0.02$) and in those with ≤ 1 salary income ($P = 0.01$) (Table 1).

IgM antibodies against *T. gondii* were found in 21/194 (10.8%) individuals, of which 9/101 (8.9%) were males and 12/93 (12.9%) were females. No association was found between seropositivity and gender, age, educational level, family income, or owning dogs and cats. All individuals who tested positive for IgM also tested positive for IgG antibodies. Females constituted 93/194 (47.9%) of individuals tested, of which 46/93 (49.5%) were of reproductive age (15–45 years old). Of these women, 18/46 (39.1%) were seronegative for *T. gondii* IgG and IgM antibodies. A total of 12/93 (12.9%) women tested positive for *T. gondii*-specific IgM, of which 7/12 (58.3%) were of reproductive age and none of them were pregnant.

Seroprevalence of specific IgG antibodies to *Toxocara* spp. and associated risk factors.

Specific IgG antibodies for *Toxocara* spp. were found in 14.4% (28/194) of subjects tested. Although not statistically significant, there was a trend towards a higher probability of *Toxocara* spp. seropositivity in children <13 years than in individuals ≥ 13 years old ($P = 0.07$; CI 0.84–5.89). A total of 124 (63.91%) of the interviewed subjects reported raising dogs, which carried no statistical significance regarding *Toxocara* spp. infection ($P = 0.08$). The majority of individuals, 89.7% (174/194) reported $\leq$ US\$300 monthly income and 57.7% (112/194) reported low levels of instruction ($P > 0.05$) (Table 2). Specific IgE antibodies to *Toxocara* spp. were found in 5.1% (10/194) individuals.

Table 1 Prevalence of *Toxoplasma gondii* IgG antibodies among individuals of Dona Carmen settlement, São Paulo, Brazil

Demographic variables	No. of subjects	No. positive (%)	OR	P	CI 95%
Gender					
Male	101	45 (44.1%)	0.50	0.02	0.28–0.90
Female	93	57 (61.3%)			
Age group					
<13 years	38	20 (52.6%)	1.03	1.00	0.49–2.0
≥ 13 years	156	82 (52.5%)			
Exposure to animals					
Raising dogs					
No	70	35 (50.0%)	0.85	0.65	0.47–1.53
Yes	124	67 (54.0%)			
Raising cats					
No	139	73 (52.5%)	0.99	1.0	0.53–1.85
Yes	55	29 (47.3%)			
Educational level					
<Elementary school	112	62 (55.3%)	1.30	0.38	0.73–2.30
$\geq$ Elementary school	82	40 (48.8%)			
Family income					
$\leq$ US\$300 monthly	174	95 (54.6%)	3.6	0.01	1.25–10.36
$\leq$ US\$300 monthly	20	7 (35.0%)			

Table 2 Prevalence of *Toxocara* spp. IgG antibodies among individuals of Dona Carmen settlement, São Paulo, Brazil, 2009

Demographic variables	No. of subjects	No. positive (%)	OR	P	CI 95%
Gender					
Male	101	11 (10.9%)	0.55	0.14	0.22–1.32
Female	93	17 (18.3%)			
Age group					
<13 years	38	9 (23.7%)	2.24	0.07	0.84–5.89
≥13 years	156	19 (12.0%)			
Exposure to animals					
Raising dogs					
No	70	6 (8.6%)	0.43	0.08	0.15–1.21
Yes	124	22 (17.7%)			
Raising cats					
No	139	18 (12.9%)	0.67	0.34	0.27–1.70
Yes	55	10 (18.1%)			
Educational level					
<elementary school	112	17 (8.8%)	0.87	0.72	0.35–2.10
≥ elementary school	82	11 (5.7%)			
Family income					
≤US\$300 monthly	174	27 (13.9%)	3.49	0.20	0.46–72.81
>US\$300 monthly	20	1 (0.5%)			

Toxoplasma gondii and *Toxocara* spp. coinfection

In this study, all the individuals who were seropositive for *Toxocara* spp. also showed anti-*T. gondii* IgG antibodies and 5.1% of them (10/194) presented *T. gondii*-specific IgM antibodies. Seropositive subjects with both anti-*Toxocara* spp. antibodies and anti-*T. gondii* IgM antibodies were invited to be further evaluated by a physician and by an ophthalmologist. None of the subjects showed ocular lesions by funduscopy.

Seroprevalence of *T. solium metacestode*-specific antibodies by ELISA-VF

Anti-*T. solium metacestode* antibodies were detected in 5.7% (11/194) of the serum samples. Amongst the interviewed subjects, 25% reported raising pigs in their backyard without access to human stool samples. Of the seropositive subjects by ELISA-VF screening test, none were seropositive in the immunoblot-18/14 kDa immunodominant peptide test.

Eosinophilia and Total IgE

Eosinophilia and increased serum IgE levels are common physiological alterations associated with

parasites such as *Toxocara* spp. and *T. solium metacestode*. Of the studied population, 27.3% (53/194) showed eosinophilia and 78.9% (153/194) showed increased levels of total IgE ($P=0.005$). No associated odds ratio (OR) was found between total IgE levels and seroprevalence of *Toxocara* spp., and *T. solium metacestode* amongst individuals from Dona Carmen settlement. However, a statistical significance was found between total IgE levels and *T. gondii* IgG antibodies (OR 1.082–4.422, CI 1.082–4.422, $P=0.042$) (Table 3). No relationship was found between *Toxocara* spp., *T. solium metacestode*, *T. gondii* IgM and IgG antibodies and eosinophils amongst individuals from the Dona Carmen settlement (Table 4).

Hematology and risk factors

Iron deficiency, i.e. anemia, is among the main laboratory findings in studies of populations with nutritional deficiency, low incomes, low education and socio-economic loss. In this study, 8.8% (17/194) individuals showed anemia (hemoglobin <11.5 g/dl), without differences in gender but with increased rates in children older than 13 years compared with those

Table 3 Total IgE antibodies odds ratio (OR) associated with seroprevalence of *Toxocara* spp., *Toxoplasma gondii*, and *Taenia solium metacestode* among individuals from the Dona Carmen settlement, Mirante of Paranapanema, Brazil, 2009

Variables	No. of subjects	No. positive (%) Total IgE	OR	P	CI 95%
<i>T. canis</i>					
Positive	39	33 (84.6%)	1.256	0.815	0.481–3.277
Negative	156	127 (81.4%)			
<i>T. solium</i>					
Positive	21	17 (80.9%)	0.863	0.757	0.211–2.214
Negative	166	143 (86.1%)			
<i>T. gondii</i> IgM					
Positive	22	18 (81.8%)	1.367	0.787	0.437–4.268
Negative	176	135 (76.7%)			
<i>T. gondii</i> IgG					
Positive	107	91 (85.0%)	2.188	0.042	1.082–4.422
Negative	90	65 (72.2%)			

younger than 13 years ($P = 0.005$). Anemia was also significantly increased ($P < 0.05$) in individuals with low salary income, using ground floor wells or rivers as a source of water supply, and those living in shacks made of plastic or canvas compared with individuals with a higher salary income, artesian wells as their source of potable water, and living in houses built with bricks.

Copro-parasitologic studies

Among 170 stool samples examined, *Entamoeba coli* cysts were present in 2.9% (5/170) and *Giardia lamblia* cysts in 2.3% (4/170) individuals. No helminthes eggs, parasites or adult proglottids were found.

Discussion

Despite the reduction in the number of deaths caused by infectious diseases in recent decades, they still constitute a public health problem worldwide, particularly in Brazil. Concomitantly, the country has experienced increasing migration of people from cities to rural areas, most of them in poor living conditions and lacking sanitary infra-structure.^{14,15} Emerging and re-emerging parasitic zoonoses such as toxocariasis, toxoplasmosis, and cysticercosis remain incontrollable in some regions.^{6,8,16} Improved and new serological and molecular tests can represent an important tool in the attenuation of morbidity and mortality due to parasitic zoonoses, especially in developing countries.

Among Dona Carmen residents, the overall seroprevalence of IgG antibodies to *T. gondii* was 52.57%. Seroprevalence rates of toxoplasmosis in Brazil have been primarily described for populations living in large cities and for women who are pregnant or of reproductive age. Little data are available for rural populations, and to our knowledge, this is the first study focusing on toxoplasmosis in a rural settlement in the western region of Brazil (Fig. 1). The seropositivity rate we observed is lower than the rates found in studies conducted among the rural populations of the Amazon region using similar methodology, which was found to be either 65.8% or

73.3%.^{24,25} Comparison of results among different populations in Brazil is difficult due to differences in the soil, climate, and spatial distribution of families, all of which definitively influence the prevalence of the parasite. Differences between the wet, rainy, and large properties in the Amazon region and the sandy, dry, and side-by-side distribution of shacks in the Dona Carmen settlement also could influence parasite prevalence. Among the risk factors associated with *T. gondii* infection, the rates of raising cats (the definitive hosts for *T. gondii*) were similar between infected and non-infected subjects. However, in communities such as Dona Carmen, stray cats and dogs can wander freely, spreading human infective-oocysts, and eggs of different parasites throughout the environment.^{1,2,15}

An increased infection prevalence rate was also found in people with $\leq$ US\$300 monthly income. Low socioeconomic status is an additional factor contributing to *T. gondii* infection in Brazilian communities and worldwide.^{7-26,27} There was also a significant increase in infections in females compared to males. This may be a result of women remaining near or inside the house, close to domestic animals, and becoming predisposed to *T. gondii* infection. In contrast, children in the settlement often stay in school, while men leave the house to work either in sugar cane plantations and distilleries or in the remaining cattle farms of the region.

IgM antibodies against *T. gondii* were present in 10.8% of the subjects. Although IgM seroprevalence is scarce in the population as a whole, this rate is one of the highest found in Brazil and elsewhere, even among pregnant women at risk of acute toxoplasmosis infection.^{28,29} The mechanism behind this result is difficult to establish as no risk factors associated with IgM infection were found. IgM antibodies are frequently the first class of antibodies detected after primary infection. A positive result in a transversal sample can be interpreted as a true-positive in acute infection, a true-positive in chronic infection, and as a false positive. The sensitivity of

Table 4 Eosinophils counts odds ratio (OR) associated with seroprevalence of *Toxocara* spp., *Toxoplasma gondii*, and *Taenia solium* metacestode among individuals from the Dona Carmen settlement, Mirante of Paranapanema, Brazil, 2009

Variables	No. of subjects	No. positive (%) eosinophils	OR	P	CI 95%
<i>T. canis</i>					
Positive	43	16 (37.2%)			
Negative	148	36 (24.3%)	1.844	0.139	0.894–3.801
<i>T. solium</i>					
Positive	12	4 (33.3%)			
Negative	173	46 (26.6%)	1.380	0.863	0.396–4.804
<i>T. gondii</i> IgM					
Positive	21	8 (38.0%)			
Negative	167	45 (26.9%)	1.668	0.416	0.648–4.292
<i>T. gondii</i> IgG					
Positive	97	27 (27.8%)			
Negative	94	25 (26.6%)	1.065	0.976	0.562–2.014

ELISA used to detect *T. gondii*-specific IgM antibodies in the acute phase is reported to be 100%. As discussed previously, the antibodies against the GRA-7 and SAG-1 antigens are considered markers of acute toxoplasmosis. Due to the high efficiency of the IgM ELISA using GRA-7 and SAG-1 antigens in detecting acute toxoplasmosis, the IgG avidity ELISA was not performed.^{11,20} As the studied population has been living in this area for at least 2 years, acute infection may be occurring. This fact is of particular importance for both pregnant women at risk of mother-to-child transmission (MTCT) and for women of gestational age who are free from *T. gondii* infection. Although none of IgM positive women indicated that they were pregnant, 18 women of reproductive age tested seronegative for toxoplasmosis. In pregnant women, a negative serological sample requires frequent investigation throughout the gestation due to the risk of the mother becoming infected and MTCT.^{28,29} In addition to helminthic infections, elevated IgE serum levels were found in many protozoal infections, however, their role in the immune response remains unclear. In this study, a relationship between *T. gondii* IgG seropositivity and IgE antibodies was found and the data were not associated with intestinal parasites since helminthes infection was not diagnosed. It was demonstrated that *T. gondii* infection affects the levels of Th2 cytokines with increased levels of IgE, IL-5, and IL-6 as compared with healthy subjects.³⁰

The seroprevalence of *Toxocara* spp. antibodies was 14.43%. There was a statistically not quite significant trend towards a higher probability of seropositivity in children aged <13 years than in individuals ≥13 years old. Young age is considered a risk factor associated with *Toxocara* spp. infection, most likely due to geophagy, onychophagy, and greater levels of contact with soil, dogs and cats. Having a dog in the household has been recognized as a risk factor for infection.¹ Although not statistically significant, 63.9% of the subjects interviewed reported having dogs, potentially contributing to infection. In rural communities, semi-wild dogs and cats wander freely and spread *Toxocara* spp. eggs over large areas, as previously discussed.^{16,31}

Both *Toxocara* spp. and *T. gondii* are known to cause ocular lesions that can lead to partial or total loss of vision in the affected eye. *Toxoplasma gondii* is considered one of the most important causes of blindness and visual impairment throughout Brazil.^{27–30} Infection with one agent could also be associated with an increased risk of infection with the other.^{26,27} In our study, 14.4% of individuals showed concomitant *Toxocara* spp./*T. gondii* infection. This was similar to a study where a 12.3% rate of *T. gondii* and *Toxocara* spp. coinfection was observed in the

children of a low income community in the outskirts of São Paulo.⁷ The presence of antibodies specific to both of these parasites was also found in 8.0% of outpatients of an Ophthalmology clinic and in 17.2% of patients suspected of toxoplasmosis infection in Egypt.^{32,33} This finding is quite different than that of a survey conducted at the Água Sumida settlement, not far from the Dona Carmen settlement, where ocular manifestations were present in a significant number of individuals.¹⁶

Antibodies specific to *T. solium metacestode* were present in 5.7% of the samples tested by ELISA-VF. Of these positive samples, all were non-reactive in the specific Western blot-18/14 kDa immunodominant peptide test. Considering the specificity of WB-18-14 kDa, this finding indicates that none of the individuals were infected. Although unexpected due to the presence of several risk factors for *T. solium metacestode* infection, one possible explanation for this result is the continuous medical assistance given by the ESF from Mirante of Paranapanema and Teodoro Sampaio Health Secretary. Corroborating with these results, in the coproparasitologic studies, there were few people infected with pathogenic cysts and none infected with helminthes among the surveyed participants. This low prevalence of intestinal parasites is in contrast with different studies carried out in rural areas and poor populations in Brazil,⁶ but are in accordance with previous surveys conducted in Pontal of Paranapanema rural settlements.^{14,16} In poor communities with both sanitary and laboratory deficiencies, prophylactic treatment with intestinal anti-parasitic drugs has become a common practice adopted by physicians.¹⁴ Furthermore, the low prevalence of intestinal parasites can explain the absence of relationship between eosinophils and total IgE levels with *T. solium metacestoda* ELISA. It is well known that *T. solium metacestoda* and *T. canis* are associated with a Th2 immune response with high levels of IgE and eosinophilia, mediated mainly by HLA class II molecules.³⁴ One possible reason for the high levels of IgE and eosinophils found was the strong allergic environment stimulus suffered by this population as shown previously in rural settlement individuals living in the surrounding area of Dona Carmen.^{14,15} Furthermore, in the studied population, an association between Th2 cytokines and *T. gondii* IgG infection was demonstrated. In conclusion, in spite of environmental, educational, and socioeconomic factors favoring parasite infection, *T. gondii*-specific IgG antibody seroprevalence was lower in the studied population than would be expected based on the rural and urban data collected from other Brazilian populations. However, the higher levels of IgM antibodies found suggest that an acute *T. gondii* infection is taking place in this community. *Toxocara*

spp. seroprevalence levels were similar to those recently found in other settlements in the region.^{15,16} None of the examined individuals showed antibodies specific to *T. solium metacestode*. Additionally, anemia, a classic laboratory marker of undernutrition and poverty, was present in few individuals. Collectively, the data validate the effort of the Brazilian Government to promote health-care services mainly through ESF assistance to poor settings communities such as Dona Carmen.

Ethical Approval

All study protocols (No. 142/08) were approved by the Ethics Committee of UNOESTE, Presidente Prudente, Brazil.

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