Toxocariasis/cysticercosis seroprevalence in a long-term rural settlement, São Paulo, Brazil

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SUMMARY

Seroprevalence of *Toxocara* and *Taenia solium* and risk factors for infection with these parasites were explored in a long-term rural settlement in São Paulo state, Brazil. An ELISA for the detection of anti-*Toxocara* IgG and IgE and anti-*T. solium* cysticerci was standardized using *Toxocara* excretory-secretory antigens (TES) obtained from the cultured second-stage larvae of *T. canis* and by vesicular fluid antigen from *Taenia crassiceps cysticerci* (VF). For cysticercosis, the reactive ELISA samples were assayed by Western blot using 18 kDa and 14 kDa proteins purified from VF. Out of 182 subjects, 25 (13·7%) presented anti-*Toxocara* IgG and a positive correlation between total IgE and the reactive index of specific anti-TES IgE ($P=0·0265$) was found amongst the subjects found seropositive for anti-*Toxocara* IgG. In these individuals 38·0% showed ocular manifestations. The frequency of anti-*T. solium* cysticerci confirmed by Western blot was 0·6%. Seropositivity for *Toxocara* was correlated with low educational levels and the owning of dogs. Embryonated eggs of *Toxocara* spp. were found in 43·3% of the analysed areas.

Key words: *Toxocara*, cysticercosis, seroprevalence, ELISA, Brazil.

INTRODUCTION

Helminth zoonoses in humans are important public health problems affecting people worldwide, especially in rural areas of developing countries, linked to poor sanitation and low economic and educational levels. Toxocariasis produced by *Toxocara canis* and *T. cati* and cysticercosis produced by *Taenia solium* are largely distributed and endemic in some Latin American regions with high sero-epidemiological prevalence of both agents (Ferrer *et al.* 2002; Nicoletti *et al.* 2002). However, in Brazil, particularly in São Paulo state, few studies have been conducted to determine both serological status and the real impact of these infections in rural sites, although these communities have been associated with a higher risk of infection. After infecting the host, *Toxocara* can reach tissues such as the liver and eye producing ocular damage and *Taenia solium* cysticerci can invade the central nervous system producing neurocysticercosis. However, *Toxocara* spp. can eventually invade the brain and *Taenia solium* cysticerci, the ocular tissue (Nicoletti *et al.* 2002; Sabrosa-Zajdenweber 2002; Hoffmeister *et al.* 2007).

The diagnosis of toxocariasis and cysticercosis are largely based on clinical examination, sero-epidemiological surveys, diagnostic images and serology since the isolation of the eggs and parasites is often difficult (Kanafani *et al.* 2006). For toxocariasis, the serological assay most commonly used is an ELISA, for detection of IgG that reacts with *Toxocara* excretory-secretory antigens (TES), although IgE and IgA are also produced. For the serological diagnosis of cysticercosis an ELISA screening test is commonly used. However, for both, although several serological methods have been proposed to date, false-negative or false-positive results have been reported, in part attributed to cross-reactivity with other cestode antigens (Ishida *et al.* 2003; Espíndola *et al.* 2005). Recently, the use of purified proteins obtained from the vesicular fluid of *Taenia crassiceps* (VF-Tcra), has successfully been reported as a source of antigens for simple tests, such as ELISA, for clinical diagnosis and surveillance of

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human cysticercosis infection. Moreover, VF-TCra is rich in low molecular weight peptides, and the purification of native specific proteins from VF-TCra *cysticerci* (18 kDa and 14 kDa), using anti-*T. crassiceps* monoclonal antibodies has been considered specific for the immunodiagnosis of cysticercosis using the Western blot assay (Bragazza *et al*. 2002; Espíndola *et al*. 2005).

In the last decade, Brazil has experienced an increasing migration of people from cities to rural areas, coordinated especially by the Landless Workers Movement (MST). Pontal of Paranapanema is one of the poorest and most undeveloped regions in the state of São Paulo, and concentrates the highest density of rural settlements found in the last decades in Brazil (Prestes-Carneiro *et al*. 2006, 2008). The Agua Sumida settlement was established in 1988 and becomes one of the main long-term settlements formed in the region. As part of the Brazilian Government's Public Health promotion effort, the Family Health Program (PSF) was introduced into this community. Our aims were to determine the seroprevalence of *Toxocara* and *T. solium* infection and explore some of the associated risk factors.

MATERIALS AND METHODS

Study design

The study was carried out in the Agua Sumida settlement located in Teodoro Sampaio County, Brazil. This area has been occupied by the MST since 1988, and was expropriated for land reform purposes by the Brazilian National Institute for Land, Settlement and Agrarian Reform (Incra). The expropriated area (4210 ha) was divided into 121 subareas (small areas of land or lots), coordinated by São Paulo's Land Institute (Itesp). The specimens were collected from December 2006 to January 2008 from 182 inhabitants, representing 15·3% of the 1163 inhabitants living in the settlement, who agreed to take part in this study. They were randomly selected among healthy volunteers of age ranging from 4 to 84 years. Prior to the recruitment, a house-by-house campaign including a folder with information about toxocariasis and cysticercosis symptoms and transmission, and the study itself was launched. The settlement has a little village in which a Public Health Centre with professionals from the PSF is situated, composed of a clinical physician, a nurse, 2 nurse assistants, a dentist, a dental assistant and 6 health agents. Their actions are widely directed towards disease prevention, health education for the community and groups at risk, and in controlling infectious disease and parasites such as dengue and worms. The subjects with positive serology for *Toxocara* or *T. solium* were examined by a general physician and an ophthalmologist from the Ophthalmology Department of Universidade do Oeste Paulista (Unoeste). All subjects included were asked to fill in an informed consent for their participation in the investigation and a short questionnaire interview was conducted including age (classified as <15 years old and $\geqslant 15$ years old), gender, place of birth, educational level, source of water supply, sanitary facilities and income, clinical manifestations compatible with toxocariasis and cysticercosis (nausea, vomiting, headache, epilepsy, convulsion, seizures and anxiety), the question of whether or not they were raising cattle, pigs, dogs or cats. The protocol of this study was approved by the Ethics Committee of Unoeste, Presidente Prudente, Brazil.

Haematology

Blood was collected in Vacutainer tubes with and without EDTA as anticoagulant. Serum was separated by centrifugation and stored at $-20\,^{\circ}$C until use. A haemogram was performed using a flow cytometer flux counter (Pentra 80, Horiba Diagnostics, Montpellier, France) and the differential leukocyte count was compared to direct microscopic observation of blood smears. The level of eosinophilia was accessed as the percentage of leucocytes represented by eosinophils (Williams *et al*. 1991).

Serology

Total IgE was determined in serum samples using an automated chemiluminescence assay (Immulite Diagnostic Products Corporation, Los Angeles, CA), and total IgA in serum samples using an automated turbidimetric assay (SERA-PAK immuno IgA, Bayer Corporation Diagnostics Division, Tarrytown, NY), according to the manufacturer's instructions, respectively. Each serum was also tested, in ELISA, for anti-TES IgG and IgE (see below).

Production of Toxocara canis *excretory-secretory antigen* (*TES*)

Antigen was prepared as described by Elefant *et al*. (2006), following a modified version of the method published by De Savigny (1975). Briefly, *T. canis* eggs were collected from the uterus of female worms obtained from dogs treated with cocoa liquor (Centro de Controle de Zoonoses de São Paulo) and, in order to embryonate, they were incubated in 2% formalin, for approximately 1 month at 28 °C. Embryonated eggs were artificially hatched in serum-free Eagle's medium, and the second-stage larvae were recovered by transferring the suspension on a loose cotton wool plug into a Baermann apparatus. After approximately 18 h, larvae were collected from the bottom of the apparatus. Cultures containing the larvae were incubated at 37 °C and, at weekly intervals, the culture fluid was removed and replaced with fresh

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medium. To the culture supernatant, constituting the antigen, protease inhibitor was added (5 μl/ml of 200 mM phenylmethylsulfonylfluoride), concentrated in an Amicon apparatus, dialysed against distilled water, centrifuged (18 500 ***g***) at 4 °C, for 60 min and filtered by 0·22 μm Millipore membrane (Millipore, Danvers, MA). The protein content was determined using the Lowry protein assay (Lowry *et al*. 1951) and the antigens were stored in aliquots at −20 °C.

Production of Ascaris lumbricoides *adult worm extracts* (*AWE*)

Non-specific antibodies were removed by serum absorption with AWE. This antigenic extract was obtained based on a previously described method (Kanamura *et al*. 1981) with modifications. In brief, *A. lumbricoides* worms were macerated in distilled water and 1·5 M NaOH was added to reach a final concentration of 0·15 M. After 2 h at room temperature, the mixture was neutralized with 6 M HCl, centrifuged (18 500 ***g***) at 4 °C, for 20 min. The protein content of the supernatant was analysed using the Lowry assay. The extract containing approximately 5 mg/ml protein was delipidized with ether (1/3, v/v).

Standardization of enzyme-linked immunoassay (*ELISA*)

The anti-*Toxocara* IgG, and IgE antibodies were detected by ELISA based on the method described by De Savigny *et al*. (1979), with some modifications (Elefant *et al*. 2006). TES antigen solution (1·9 μg/ml in a 0·1 M carbonate-bicarbonate buffer, pH 9·6) was used to load the polystyrene plates (Corning Incorporated-Costar, New York USA). The blocking solution for IgG was 1% bovine-serum-albumin (BSA, Sigma, USA) in 0·01 M phosphate-buffered saline pH 7·2, containing 0·05% Tween 20 (PBS-T) and, for IgE-ELISA, 5% skim-milk (Molico, Nestlé). All sera were pre-absorbed with AWE (25 μg/ml) in PBS-T, for 30 min at 37 °C. Serum samples were diluted 1/320 for IgG-ELISA, and 1/50 for IgE-ELISA. In the IgE-ELISA, serum samples were also pre-absorbed with AWE (25 μg/ml) and with RF-Absorbent v/v (Dade Behring). Peroxidase conjugates were used as follows: 1/10 000 dilution of anti-human IgG (Sigma, USA), and 1/500 dilution of anti-human IgE (Sigma, USA). As a chromogen substrate, ortho-phenylenediamine (0·4mg/ml, OPD-Fast, Sigma, USA) and H_2O_2-urea (0·4 mg/ml), in 0·05 M citrate buffer were added. The reaction was stopped with 2 M H_2SO_4. The assay was monitored by including negative and positive serum samples. Absorbance at 492 nm was determined in an automatic microplate reader (Titertek Multiskan MCC/340, Lab-system, Finland). The cut-off values were calculated based on mean absorbance of 96 negative serum controls determined prior to the study ±3 standard deviations for IgG-ELISA (0·300) and ±2 standard deviations for IgE-ELISA (0·220). Reactivity index (RI) values were calculated by dividing the absorbance value of the sample by the cut-off value. Samples with RI > 1 were considered positive. Using human serum samples with visceral toxocariasis, the authors found a sensitivity of 100% and specificity of 82·3% for IgG, and 78·3% for IgE respectively (Elefant *et al*. 2006). In an experimental model (Ishida *et al*. 2003), rabbits immunized with excretory-secretory components of *T. canis* larvae antigens (TES) showed in ELISA assay, sensitivity of 100% and specificity of 82·3%.

T. solium *enzyme linked immunosorbent assay* (*ELISA-VF*)

Anti-*T. solium* cysticercus antibodies were detected by ELISA, carried out as described by Bueno *et al*. (2000) with some modifications. The antigen was vesicular fluid (VF) from *Taenia crassiceps* cysticerci obtained as reported by Vaz *et al*. (1997). Each well of 96-well ELISA polystyrene high binding plates (Costar Corning Inc., Cambridge, MA, USA) was coated with 100 μl of antigen (0·5 μg/ml) in 0·5 M carbonate-bicarbonate buffer (pH 9·6) for 18 h in a humidified chamber at 4 °C. The wells were blocked for 1 h with 5% milk in phosphate-buffered saline (PBS; 0·01 M, pH 7·2; 0·0075 M Na_2HPO_4, 0·025 M NaH_2PO_4, 0·15 M NaCl) containing 0·05% Tween 20 (PBS/T), and then incubated for 1 h with serum samples diluted 1 : 50. Goat anti-human IgG peroxidase-conjugated (Biolab Diagnóstica S. A., Rio de Janeiro, Brazil) was added and plates were incubated for 1 h. After each incubation step, the plates were washed using an automatic washer, with 4 cycles of saline (0·15 M NaCl) containing 0·05% Tween 20. Ortho-phenylenediamine (1 mg/ml) and H_2O_2 (1 μl/ml) diluted in 0·2 M citrate buffer (pH 5·0) were added (in the dark) as chromogenic substrate and plates were incubated for 20 min. The reactions were stopped by adding 100 μl of 2 M H_2SO_4. Colour intensity was quantified using an ELISA plate reader (Diagnostics Pasteur, Strasburg-Schiltigheim, France) at 492 nm. All incubations were carried out at 37 °C. The authors reported a sensitivity of 91·2% and a specificity of 80·0% for this ELISA using serum samples (Vaz *et al*. 1997). In another study that tested 1863 human serum samples, 92·0% sensitivity and 96·4% specificity was found (Bragazza *et al*. 2002).

Western blot (*WB-18/14*)

The ELISA-reactive samples were submitted to Western blot assay using 18/14-Tcra proteins for the

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analysis of antibody specificity. The 18/14-Tcra proteins were separated by sodium duodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) following the method described by Laemmli (1970). The antigens were solubilized with sample buffer (0·01 M Tris-HCl, pH 6·8, containing 2% SDS, 5% 2-mercaptoethanol and 10% glycerol) at 100 °C for 5 min and separated eletrophoretically in 15% polyacrylamide gels. The separated antigens were eletrophoretically transferred to a 0·22 μm pore-size membrane of polyvinylidene difluoride (Millipore Corp., Bedford, MA, USA). The membranes were blocked by treatment with 5% skimmed milk (Molico Skim milk, Nestlé, Araçatuba, SP, Brazil) in PBS for 2 h, washed in PBS containing 0·05% Tween 20 (Merck, Schudart, Munich, Germany), and then incubated for 18 h at 4 °C with serum diluted at 1 : 50, in 1% skimmed milk in PBS. After further PBS washes, strips were incubated for 1 h with goat anti-human-IgG-biotin/avidin-peroxidase (Sigma Chem. Co., St Louis, MO, USA) conjugate. After additional washes, 4-chloro-1-naphthol (Sigma) predissolved in methanol (20% of the volume) and then diluted to 0·05% with Tris-buffered saline (0·01 M Tris, 0·15 M NaCl, pH 7·4) containining 0·06% H_2O_2 was added as chromogenic substrate.

Cross-reactivity of human sera infected with different helminths, as well as immunized rabbit sera, with *T. solium* and *T. crassiceps* was demonstrated using ELISA and WB. Very low or no cross-reaction was observed with other helminths tested. Furthermore, cross-reactivity between cysticercosis and toxocariasis did not occur (Ishida *et al.* 2003).

Soil samples

Since soil contamination with embryonated eggs is the main source of human *Toxocara* infection, we investigated the presence of eggs in soils of Agua Sumida settlement. Soil samples were collected in 15 lots where at least 1 inhabitant had shown antibodies against *Toxocara* and in 15 lots where the results of ELISA were negative in the whole family, totalling 30 (24·8%) out of the 121 lots in the settlement. About 100 g of soil was collected from each lot as follows: 2 samples in front of the house, 2 from each side, and 2 from behind the house. The samples were taken to Presidente Prudente and stored in the refrigerator until they were processed as described by Nunes *et al.* (1994). As each sample was checked 3 times, there were 24 eggs counts for each of the sampled lots.

Ophthalmological examination

Patients with positive IgG to *Toxocara* were examined by a general clinician followed by an ophthalmologist and underwent indirect fundoscopy ocular examination by mydriatics effects (tropicamida and fenilefrine).

Statistical analysis

An independent samples *t*-test was used to compare *Toxocara* IgG antibody level with the presence of dogs, family income or education level. When indicated, chi-square (χ^2), Fisher Exact and the correlation coefficient tests were applied using GraphPad software (V4.0) (San Diego, CA, USA) and SigmaStat software (Systat Software Inc., Richmond, CA, USA). The multivariate analysis was performed using the BioEstat software system (Sociedade Civil Mamirauá, MCT – CNPq, Belém, Pará, Brasil) (Ayres *et al.* 2007). Multivariate odds ratio (ORs) with 95% confidence intervals (CIs) were estimated by means of multiple logistic regression analysis. The statistical significance (5%) of differences in gender-, age-, risk factor-, educational level-, family income-, and clinical symptom seropositive rates among comparison groups was examined by testing the statistical significance of the regression coefficients.

RESULTS

Population characteristics

Between December 2006 and February 2008, 182 out of 1163 (15·6%) inhabitants of Agua Sumida settlement participated in this survey, from which 64 (35·0%) were male and 118 (65·0%) were female (Table 1). The female to male ratio was 1·8 : 1. The age distribution ranged from 4 to 86 years, with a mean age of 34·5 years (95% CI 32·8 to 38·9 years), of which 44 (35·2%) aged <15 years and 138 (64·8%) aged $\geqslant 15$ years. Eighty-seven (47·8%) of the individuals were born in São Paulo state, 19 (10·4%) were born in Paraná state, 11 (6·0%) in Minas Geraes state, 10 (5·5%) in Ceará state and 29 (15·9%) were born in different states mainly of North-east of Brazil. The number of individuals born in São Paulo state was similar to that of individuals that came from other states ($P > 0·05$). Water supply is provided to the lots mainly by communitarian artesian or by ground floor wells, called 'cacimbas', equipped with electric motors. All individuals interviewed were reported to have a potable water supply.

Haematology

Haematological abnormalities are among the most common manifestations in nematode infections. From the 176 individuals analysed, mild anaemia occurred in 6 (2·4%) individuals, classified as haemoglobin $<11·5$ g/dl. The mean values $\pm$ S.E.M. with 95% CI were: red blood cells (M/μl) $4·81 \pm 0·03$ (4·74–4·88); haemoglobin (g/dl) $14·0 \pm 0·09$ (13·9–14·2); haematocrit (%) $41·0 \pm 0·25$ (40·5–41·6); MCV

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Table 1. Multivariate odds ratios for various risk factors associated with seropositivity of antibodies to *Toxocara* among individuals from the Agua Sumida settlement, São Paulo, Brazil

Variable group	No. tested	No. positive (%)	*P*	Multivariate odds ratio	CI 95%
Gender					
Male	64	8 (12·5%)			
Female	118	17 (14·4%)	0·6719	0·8037	0·29–2·21
Age group					
<15 years	44	9 (20·4%)			
⩾15 years	138	16 (11·6%)	0·2908	0·5720	0·2–1·61
Risk factors					
Raising dogs					
No	17	0			
Yes	165	25 (15·1%)	0·8151	7691·0834	0–∞
Raising cats					
No	67	5 (7·5%)			
Yes	115	20 (17·4%)	0·1729	2·1729	0·71–6·63
Sanitary facilitties					
Cesspool	161		0·6813	0·56	0·04–8·91
Sewer	21		0·8579	0·8066	0·08–8·48
Educational level					
Illiteracy	26	5 (19·2%)	Ref		
Elementary school	112	17 (15·2%)	0·2677	0·4983	0·15–1·71
High School	38	3 (7·9%)	0·0884	0·2289	0·04–1·25
University	6	0	0·8797	0·0001	0–∞
Family income					
U$ <150	31		Ref		
U$ >150<300	106		0·9727	1·0234	0·27–3·84
U$ >300	45		0·4785	1·7116	0·39–7·56
Clinical symptoms					
Headache	121	16 (8·9%)	0·0321	3·0039	1·1–8·21
Seizures	67	8 (4·4%)	0·7002	0·8111	0·28–2·36
Nausea	47	11 (6·1%)	0·9212	0·9494	0·34–2·65

(fl) 85·5±0·45 (84·6–86·5); MCH (pg) 29·3±0·15 (29·0–29·6); MCHC (g/dl) 34·2±0·08 (34·0–34·4); platelets (Kμl) 237,1±4·71 (227·7–246·4). When the main alterations of white blood cells were analysed in the whole population, 17 (9·7%) showed leucopaenia, 13 (7·4%) leukocytosis, 25 (14·2%) neutropaenia, 41 (23·3%) eosinophilia, 18 (10·2%) lymphocytosis and 11 (6·3%) monocytosis. Thrombocytopaenia was present in 9 (5·1%) of the individuals.

Toxocara

The frequency of IgG anti-*Toxocara* indicated by IgG antibodies was 13·7% (25/182), with no differences in seroprevalence between genders in individuals 15 years of age or less and in those older than 15 years (Table 1). Multivariate analysis showed that persons that raised dogs had a higher risk for infection of *Toxocara* compared with those without such a history (OR=7·691, 0·8% CI=infinite), respectively. Individuals with a higher educational level had fewer possibilities of *Toxocara* infection compared with illiteracy individuals (OR=0·0001% CI=infinite). Interestingly, headache was the main clinical symptom shared by infected individuals compared with seizures and nausea (OR=3·00 CI=1·1–8·21, 0·81 CI=0·28–2·36 and 0·94 CI=0·34–2·65), respectively. From the 176 individuals investigated for total IgE, 126 (71·5%) had elevated levels of antibodies. Among the 25 subjects found to be seropositive for anti-*Toxocara* IgG, there was a positive correlation between the levels of total IgE and the reactivity index for specific anti-TES IgE ($P=0·0265$, $r=0·437$). Forty-one (21·4%) of the subjects had elevated eosinophil levels. Increased levels of total IgA antibodies were found in 13 (7·4%) of the individuals. No correlation with total or specific IgE, or with eosinophils, was found. When leucocyte alterations were compared between individuals found to be positive for *Toxocara* IgG antibodies and those with negative serology, a significant difference in white blood cell counts with increases in neutropaenia, lymphocytosis, monocytosis and eosinophilia was found in *Toxocara*-infected individuals (Fig. 1). From the 25 individuals with anti-TES IgG antibodies, 21 (84·0%) were examined by a general clinician and an ophthalmologist and the main manifestations were: ocular lesions in 8 (38·0%), onicophagy in 5 (23·8%) and hepatomegaly in 4 (19·0%) (Table 2).

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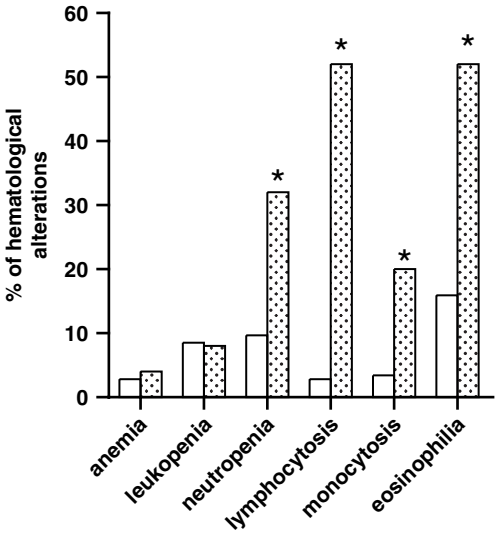

Fig. 1. Haematological alterations in haemograms from individuals that resulted positive for *Toxocara* antibodies (spotted bars) compared with individuals that resulted negative (open bars). * $P=0{\cdot}01$ for neutropaenia, * $P=0{\cdot}0001$ for lymphocytosis, * $P=0{\cdot}01$ for monocytosis and * $P=0{\cdot}0007$ for eosinophilia.

Embryonated eggs in soil samples

Embryonated *Toxocara* spp. eggs were found in 13 (43·3%) samples, although no difference was found between the contamination frequency in lots with reported occurrence of *Toxocara*-seropositivity referred to as anti-TES IgG antibodies, and in lots with no record of *Toxocara*-seropositivity.

Taenia solium *ELISA-VF*

In Agua Sumida subjects, from 179 serum samples tested, 4 (2·2%) presented cut-off >0·350.

Western blot (*WB-18/14*)

From the 4 positive samples in ELISA-VF, 1 (0·6%) was strongly reactive in the WB-18/14 kDa (Fig. 2). Considering the specificity of the results of the WB-18-14, the frequency of positivity for anti-cysticercus antibodies in the population of Agua Sumida settlement, was 0·6%. A 32-year-old man, born in Paraná state, an endemic area for *T. solium* infection showed, concomitantly, anti-*T. solium cysticercus* antibodies and positive Western blot and anti-*Toxocara*, as well as increased total and specific IgE antibodies levels. Although he reported persistent vertigo he did not adhere to clinical examination and refused to be followed by a neurologist.

Among the subjects interviewed, 29·7% (54/182) claimed to raise pigs in their lots.

DISCUSSION

The overall seroprevalence for infection with *Toxocara* referred as anti-TES IgG antibodies among the individuals of Agua Sumida settlement was 13·7%. Among the subjects found to be seropositive there was a positive correlation between the levels of total IgE and the reactive index for specific anti-TES IgE. Eosinophil counts were increased in seropositive compared with seronegative individuals. Besides, raising dogs and educational level are risk factors associated with *Toxocara* infection in this population.

Anti-*T. solium* cysticercus antibodies detected by ELISA-VF were found in 2·2% of the subjects and 1 (0·6%) was immunoreactive to the immunodominant WB-18-14 kDa peptides. Hence, considering the specificity of the results of WB, the frequency of positivity for anti-cysticercus antibodies in the population of Agua Sumida settlement was 0·6%. The prevalences of anti-*Toxocara* and anti-*T. solium cysticercus* were <21·5% and <5·9%, respectively, in individuals from the settlement of Padre Josimo, an area occupied recently by MST and located in the surrounding area of Agua Sumida (Prestes-Carneiro *et al*. 2006, 2008). Of note, however, is that the Padre Josimo settlement has no Public Health Care Centre and PSF professionals. Created in 1988, Agua Sumida has a long-term effort of Public Government and private institution investments in programmes designed to improve the access and quality of local

Table 2. Non-specific clinical manifestations in individuals with anti-*Toxocara* antibodies

Manifestations	Number	Percentage
Cutaneous	3	14·3
Pulmonary	1	4·8
Hepatic	4	19·0
Onicophagy	5	23·8
Ocular	8	38·0
Assymptomatic	4	19·0

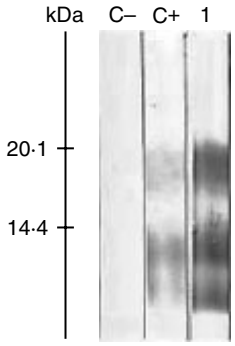

Fig. 2. Results of Western blot using 18/14-Tcra antigen purified by anti-vesicular fluid-*Taenia crassiceps* monoclonal antibody, with serum samples from a healthy individual (C−); serum sample from a patient with neurocysticercosis (C+); and serum sample from an individual from the Agua Sumida settlement (1).

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health and basic household infrastructure services, including potable water supply and sanitation as well as an improved Educational System. Poor sanitation and lack of educational level are risk factors directly associated with *T. solium* and *Toxocara* transmission in Brazil and elsewhere (Alderete *et al*. 2003; Kanafani *et al*. 2006).

Although the Ministry of Health recommends compulsory notification, few states imposed programmes to combat and control taeniasis-cysticercosis. In São Paulo state the impact of the disease is underestimated. Another difficulty is the heterogeneity of the methods for investigation, and the available data are obtained from sero-epidemiological surveys, clinical studies and case-reports. In Agua Sumida settlement, even if 21·7% reported the raising of pigs in their lots, a low frequency of individuals with anti-cysticercus antibodies was found, compared with 2·2% in São Paulo state and 2·3% in the whole country (Agapejev, 2003). Aggravating risk factors that may contribute to the endemic nature of taeniasis-cysticercosis show a strict association especially with human hygiene, including source of water supply, bathroom facilities, schooling and the raising of pigs. Thus, families that produce domestic pork in Agua Sumida are recommended not to allow pigs to roam at will, rooting and scavenging. All interviewed individuals reported having a potable water supply that was provided mainly by communitarian artesian floors. Eighty-five percent were associated with elementary school, high school or university and 100% of those interviewed stated having a close sewage system, cesspool or restroom in their houses. Bragazza *et al*. (2002) reported an anti-cysticercus serum prevalence of 2·1% in individuals from a rural population in São Paulo state, highly associated with contamination of the drinking water. Sato *et al*. (2006) demonstrated that taeniasis and cysticercosis are endemic in an important rural pork production region in which pigs are raised wandering freely in the streets.

Another important issue that certainly widely contributed to the lower seroprevalence of these nematodes in Agua Sumida subjects is the presence of the Public Health Centre and PSF professionals. Addressed to function as a primary health-care centre they also provide hygiene, health promotion/illness prevention activities, working along with the families, schools and rural associations. It is well established that the contribution of the health professionals and services are highly important to the ongoing livelihood and social infrastructure of fragile rural communities (Farmer *et al*. 2003). It should be noted that addressing the importance of health education in helminth zoonosis prevention, even if 91·0% of the individuals reported to own dogs and 63·0% to own cats, the main definitive hosts responsible for transmission of toxocariasis to humans, the seroprevalence of 13·7% is lower than that reported by sero-epidemiology surveys in Brazil (Vicente *et al*. 2005; Figueiredo *et al*. 2005; Paludo *et al*. 2007). Furthermore, 43·0% of the lots analysed contained embryonated eggs of *Toxocara* but, similarly to our previous findings, a sampled lot in which a seropositive subject lived was no more likely to be found positive for *Toxocara* eggs than a sampled lot in which no seropositives had been detected (Prestes-Carneiro *et al*. 2008; Santarém *et al*. 2008).

While pork meat ingestion and poor sanitation are linked to the transmission routes of taeniosis-cysticercosis diseases, the environmental contamination is more frequently associated with human toxocariasis. However, in both situations, as a consequence, an intricate immune response is triggered by immunocompetent hosts, *broadly* different from one person to another (Sotelo, 2003). Among the subjects found to be seropositive for anti-*Toxocara* there was a positive correlation between the levels of total IgE and the reactive index for specific anti-TES IgE. In addition, eosinophil counts were increased in seropositive compared with seronegative individuals. Interestingly, we found a significant increase in neutropaenia, lymphocytosis and monocytosis in individuals that resulted positive for *Toxocara* antibodies compared with negative individuals. Recently, Hoffmeister *et al*. (2007) also demonstrated increased leukocyte counts and eosinophilia in a patient with cerebral toxocariasis induced by raw duck liver ingestion.

When the individuals with anti-TES IgG antibodies were examined by a general clinician and by an ophthalmologist the main manifestations were ocular lesions, onicophagy and hepatomegaly. *Toxocara* is a well-documented cause of intraocular inflammation induced by ocular larva migrans (OLM) producing uveitis, posterior and peripheral retinochoroiditis, vitritis, endophthalmitis, papilitis and other ocular lesions leading to partial or total loss of vision in the affected eye (Sabrosa-Souza, 2001; Logar *et al*. 2004). Our results for prevalence in ocular lesions were similar to those found by Altcheh *et al*. (2003) but higher than those found by Lopes *et al*. (2005), determined in children with positive serology for *Toxocara* in Argentina.

In conclusion, the detection of anti-*Toxocara* and anti-*T. solium cysticercus* antibodies in the long-term Agua Sumida settlement subjects were lower than those found in a recently formed settlement, located in the surrounding area. We suggest that these results could, in part, be the consequence of continued investment of public and private institutions destined to improve education, income and infrastructure services, as well as the role of PSF in health-care and prevention.

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