

Taeniosis-cysticercosis complex in individuals of a peasants' settlement (Teodoro Sampaio, Pontal of Paranapanema, SP, Brazil)

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In order to evaluate the taeniosis-cysticercosis complex in a population of a peasants' settlement, located at Teodoro Sampaio, state of São Paulo, Brazil (longitude 52°36'12", latitude 22°17'12") a series of laboratory markers were determined. After signing an informed consent, participants answered a standardized questionnaire. To determine anti-Taenia solium cysticercus antibodies, the samples were tested by enzyme linked immunoabsorbent assay using 18-and 14-kDa antigen proteins from vesicular fluid of Taenia crassiceps (VF-Tcra). The reactive and inconclusive ELISA samples were tested by immunoblotting. Total IgE levels were determined by chemiluminescence's assay and hemogram by flow cytometer flux counter. A total of 84 individuals, 5.9% presented anti-T. solium cysticercus antibodies in ELISA and 3.6% were strongly reactive in the 18/14 kDa immunoblotting confirmatory test. All of the individuals with positive antibodies showed elevated Total IgE levels. We conclude that the frequency of anti-T. solium cysticercus antibodies in this population is higher than other regions considered endemic in São Paulo. Thus, it is important to carry out surveys in Peasants' settlement areas with the objective of establishing public health measures for prevention and control of infectious diseases such as taeniosis-cysticercosis.

Key words: *Taenia solium* cysticercus antibodies - enzyme linked immunoabsorbent assay - immunoblot - IgE - Brazil

The taeniosis-cysticercosis complex is an important cause of morbidity and mortality, widespread not only in most underdeveloped countries, but also in industrialized ones (Raether & Hanel 2003). There is strong evidence supporting the high prevalence of *Taenia* infection in humans from resource-poor areas, especially in rural areas with deficient sanitation, low hygienic standards, and unusual customs, such as consumption of raw pork (Phiri et al. 2003).

Taenia solium and *Taenia saginata* are two taenids of great economic and medical importance, causing bovine and porcine cysticercosis and taeniosis in humans. The encysted larva, known as cysticercus from *T. saginata* and *T. solium* are found in cattle and swine, respectively, and the adult tapeworm of *T. saginata* and *T. solium* are found in man. The cycle is completed when eggs in proglottids shed from the human tapeworm are disseminated to the environment through feces, followed by the ingestion by cattle or swine, and then the viable cysts are eaten by man in undercooked beef (Lees et al. 2002). People are also infected by ingestion of *T. solium* or *T. saginata* eggs by contact with carriers or contaminated foods.

Cysticercosis is an infection caused by cysticercus, the larval stage of Cestoda Family Taeniidae. Neurocysticercosis (NC), the nervous-system form of cysticercosis is caused by larvae of tapeworm *T. solium* (Carpio 2002).

In Brazil, several previous studies have shown that NC in humans is an important public-health problem; although, very few reports on the prevalence and distribution of porcine cysticercosis have been published (Sakai et al. 2001). In the state of São Paulo, the frequency of anti-*T. solium* cysticercus antibodies in humans was determined in few counties (Vaz et al. 1990, Bragazza et al. 2002). Nonetheless, studies about the prevalence of *T. saginata* infection in humans or the presence of *T. saginata* cysticercus in cattle are scarce (Ungar & Germano 1992). Dias et al. (1991) found in 311 samples of stools of humans living in the state of São Paulo an occurrence of 0.5% in *Taenia* sp. eggs; of which 273 were identified as *T. saginata* proglottidis.

The ELISA screening test is commonly used for the serological diagnosis of cysticercosis, although it cross-reacts with cestoda parasites such as *Hymenolepis nana* and *Echinococcus granulosus* (Garcia et al. 2003, Ishida et al. 2003). Low-molecular-weight glycoproteins obtained from *T. solium* by affinity chromatography, considerably improved the specificity of ELISA screening tests, but a large amount of antigens is required (Tsang et al. 1989). Recently, it has successfully been reported the use of low-molecular-weight peptides obtained from the vesicular fluid of *T. crassiceps* antigens (Tcra-VF) and *T. crassiceps* cysticercus glycoproteins (Tcra-GP and

Tcra(18-14)-GP in ELISA and immunoblot assays (Bueno et al. 2000, Pardini et al. 2002, Ishida et al. 2003).

The Pontal of Paranapanema region is located in the west of the state of São Paulo and concentrates the highest number of "Landless Peasants' Settlements" of the country. The region is characterized by large raising cattle farmers, and in the last decades, the "Landless Peasants" population increased abruptly, and pressure on proper land resulted in a large number of "Landless Peasants' Settlements". They are coordinated by the "Landless Peasants' Movement" (Itesp 2004).

These settlements are usually settled on marginal lands or beside highways, near cattle raising areas and water sources. Poor living conditions and lack of sanitary infrastructure, characterized by the use of open areas and fields for defecation, represent an important source of environmental contamination, which in turn may establish the biologic life cycle of parasites such as *Taenia* sp.

In order to evaluate the *Taeniase-cysticercosis* complex in Pontal of Paranapanema region, we determined laboratory markers in individuals from Pe. Josimo Peasants' settlement, located in Teodoro Sampaio, São Paulo, Brazil.

MATERIALS AND METHODS

Study design - Pe. Josimo Settlement, located in Teodoro Sampaio county, in the west region of the state of São Paulo, Brazil (longitude 52°36'12", latitude 22°17'12") is an area occupied by the "Landless Peasants' Movement" since 2000, and recently expropriated for land reform purposes by the Brazilian National Institute for Land, Settlement and Agrarian Reform (Incra). With a tropical climate, followed by a rainy season in the summer, the region has a dry weather in the winter with a sandy top-soil mostly meant for pasture and cattle raising, with temperatures ranging from 28°C to 36°C in the summer. Most of the individuals interviewed lived in the marginal land, in a camping of plastic huts from 2000 to 2003, when the whole-expropriated area (2290 he) was divided into 96 small areas coordinated by São Paulo's Land Institute (Itesp 2004). The specimens were collected from February to April 2004, among 84 (25.8%) out of 325 inhabitants from Pe. Josimo Peasants' settlement that agreed with this research. The results were set out for Teodoro Sampaio's Municipal Health Department, and individuals with anti-*T. solium* cysticercus reactive on ELISA and confirmed by immunoblot were directed to a neurologist of Hospital Universitário "Domingos Leonardo Cerávolo", Presidente Prudente, SP. The protocol of this study was approved by the Ethical Committee of Universidade do Oeste Paulista (Unoeste) no. 001/04, Presidente Prudente, SP, Brazil.

Twenty serum samples from patients with NC were tested. These patients had NC diagnosis confirmed by imaging exam (CT and/or MRI), clinical and immunological data, and were obtained from patients being seen at the Medicine Faculty Hospital of the University of São Paulo, São Paulo, Brazil. The control group consisted of 194 serum samples from apparently healthy individuals.

Data collection - Data were collected using a standardized questionnaire that included age, gender, place of birth, educational level, source of water supply, sani-

tary facilities, income, clinical manifestations compatible with neurocysticercosis, and raising of swine and cattle.

The blood was collected in Vacutainer tubes with and without EDTA as anticoagulant. Serum was separated by centrifugation and stored at -20°C until use.

Antigen - The 18- and 14-kDa proteins from vesicular fluid of VF-Tcra purified with monoclonal antibody anti-Tcra (18/14-Tcra) were obtained as described by Espindola et al. (2005).

Enzyme linked immunoabsorbent assay (ELISA-18/14) - Anti-*T. solium* cysticercus were detected by ELISA assay, carried out as described by Bueno et al. (2000) with some modifications. The test was carried out using 18- and 14-kDa proteins. Each well of 96-well ELISA polystyrene high binding plates (Costar Corning Inc., Cambridge, MA) 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; 0.01M; pH 7.2; 0.0075 M Na₂HPO₄, 0.025 M NaH₂PO₄, 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 four cycles of saline (0.15 M NaCl) containing 0.05% Tween 20. Orthophenylenediamine (1 mg/ml) and H₂O₂ (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 H₂SO₄. Color intensity was quantified using an ELISA plate reader (Diagnostics Pasteur, Strasburg-Schiltigheim, France) at 492 nm. All incubations were carried out at 37°C.

Immunoblotting (WB-18/14) - The ELISA reactive samples were submitted to immunoblotting using 18/14-Tcra proteins for the analysis of antibody specificity. The 18/14-Tcra proteins were separated by sodium dodecyl 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 electrophoretically in 15% polyacrylamide gel. The separated antigens were electrophoretically transferred to a 0.22-µm-pore-size membrane of polyvinylidene difluoride (Millipore Corp., Bedford, MA). 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 sera 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) conjugate. After additional washes, 4-chloro-1-naphthol (Sigma) pre-dissolved 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) containing 0.06% H₂O₂ was added as chromogenic substrate.

Total IgE levels - Total IgE was determined in sera samples using an automated chemiluminescence assay (Immulite Diagnostic Products Corporation, Los Angeles, CA), according to the manufacturer's instructions.

Hemogram - The hemogram was undertaken by a flow cytometer flux counter (Pentra 80, Horiba Diagnostics, Montpellier, France), and the leukocyte differential counting was compared to direct microscopic observation in blood smears, taken as the reference method. Eosinophils counts were classified as proposed by William's (1991).

Coproparasitologic studies - The stools were collected and examined using Faust and Hoffmann, Pons and Janner methods, for detection of cysts, parasites or adult proglottidis (Pessoa & Martins 1982).

Statistical analysis - Anti-*T. solium* cysticercus and IgE levels were compared using independent samples t test and chi-square (χ^2) and Fisher Exact Method were applied when indicated through the GraphPad Software (V3.0) (San Diego, CA). The level of statistical significance was 5%.

RESULTS

Demographic, environmental and behavioral characteristics - From Pe. Josimo peasants' settlement inhabitants that participated in this survey, 38 (45.2%) were female and 46 (54.8%) were male. The population age distribution ranged from 6 to 71, of which 55 (65.5%) were classified as adults and 29 (34.5%) as children. Forty-two (50%) of the individuals were born in São Paulo, 11 (13.1%) were born in Paraná, and 33 (39.2%) in other Brazilian states. Only 6 (7.1%) had high school education and 10 (11.9%) lacked elementary instruction. The majority (77.3%) had completed primary school. The income varied from less than US\$ 100.00 (91.6%) to about US\$ 170.00 (8.4%) per month. Seventy-four individuals (88%) had ground floor wells or "cacimbas", and 10 (11.9%) used opened ground mines as water source. Seventy-eight individuals (92.8%) have latrines located beside the houses and 6 (7.1%) have neither toilet facilities nor latrines, and defecated outdoors. Twenty-one (25%) had pig husbandry and 47 (55.9%) domestic cattle. Among the interviewed individuals 24 (28.6%) reported having headaches, and 10 (11.9%) vertigo. None of them reported having convulsion or epilepsy.

ELISA-18/14 - The analysis of 20 serum samples from patients with confirmed NC showed sensitivity of 100% (Fig. 1). The normal serum samples from 194 healthy individuals presented cut-off borderline ≤ 0.350 nm and specificity of 99.5% (Fig. 1). In Pe. Josimo Settlement group, from 84 serum samples tested, 5 (5.9%) samples presented cut-off ≥ 0.350 nm (Fig. 1).

Immunoblotting (WB-18/140) - From 5 (5.9%) samples from Pe. Josimo settlement group that presented cut-off ≥ 0.350 nm in the ELISA-Tcra 3 (3.6%) were strongly reactive in the IB-18/14 kDa (Fig. 2). Serum samples from controls with negative and positive ELISA results were ana-

lyzed by IB-18/14, and found to be positive (see 1 to 3 in Fig. 2) and negative (see 4 and 5 in Fig. 2), respectively.

Total IgE - Seventy-eight (93.9%) out of 83 serum samples showed Total IgE concentration higher than normal values (Fig. 3).

We found a positive association in total IgE levels and the anti-*T. solium* cysticercus antibodies ($*p < 0.05$), since 5 (100%) of the samples that presented ≥ 0.350 nm cut-off showed elevated levels of IgE distributed as follow: mild 1 (20%), moderate 3 (60%), and severe 1 (20%).

Hemogram - Of the 81 individuals submitted to hemogram counts 65 (80.2%) showed isolated or associated erythrocyte/leukocyte disorder. Thirty individuals (37%)

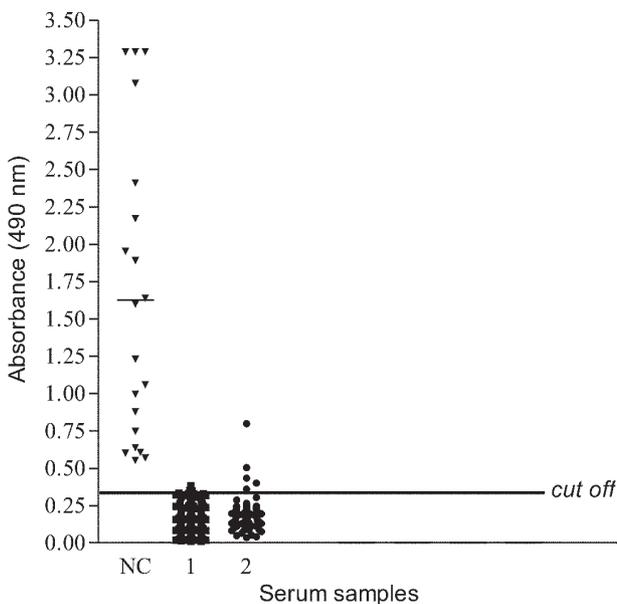


Fig. 1: enzyme linked immunoabsorbent assay results with vesicular fluid-*Taenia crassiceps* antigen (18/14-kDa proteins) purified with anti-VF-Tcra, of serum samples from patients with neurocysticercosis (NC), serum samples from apparently healthy individuals, (1) and serum samples from individuals of Pe. Josimo Peasants' settlement (2).

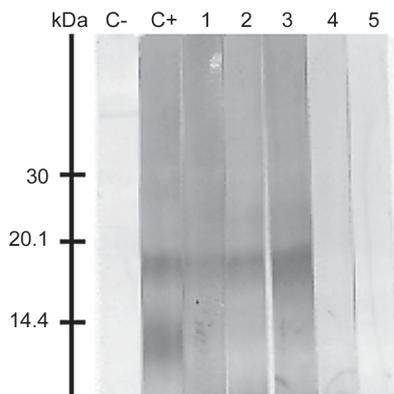


Fig. 2: results of immunoblotting using 18/14-Tcra antigen purified by anti-vesicular fluid-*Taenia crassiceps* monoclonal antibody with serum samples from apparently healthy individuals (C -); serum sample from patients with neurocysticercosis (C +); and serum samples from individuals of Pe. Josimo peasants' settlement (1, 2, 3, 4, and 5).

presented enhanced eosinophils counts distributed mainly in children: 0|–10 years of age 5 (16.6%), 10|–20, 7 (23.3%), and old people ranging from 50|–60, 6 (20%), and 60|– years of age 5 (16.6%). We found a positive association in the whole studied population when correlating IgE levels with increased eosinophils counts ($*p = 0.02$) the mean \pm SEM of IgE levels in normal, mild and moderate eosinophils group was: 618.8 ± 102.9 ; 922.5 ± 121.3 ; and 1671 ± 322.5 , respectively (Fig. 3). However, it was not found association between anti-*T. solium* cysticercus antibodies and eosinophilia.

Coproparasitologic studies - Among 49 stools examined, *Entamoeba coli* cysts were present in 9 (18.3%) and *Giardia lamblia* cysts in 3 (6.1%) individuals.

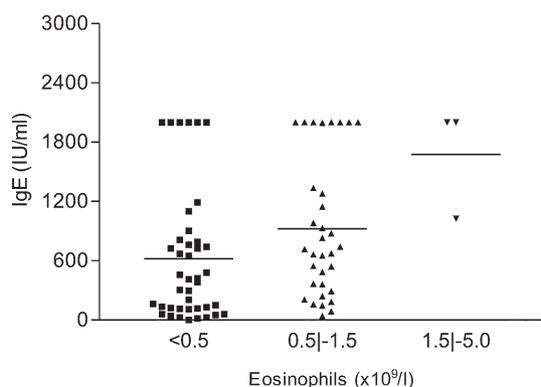


Fig. 3: correlation between IgE levels and eosinophils in individuals from Pe. Josimo settlement, Teodoro Sampaio, SP, Brazil. The eosinophils were classified in the hemogram as: normal $< 0.5 \times 10^9/l$; mild $0.5\text{--}1.5 \times 10^9/l$; moderate $1.5\text{--}5.0 \times 10^9/l$ in 84 individuals. Total IgE levels were determined by chemiluminescence method in 83 serum samples and classified according to our own reference ranges as follows: normal (< 100 IU/ml); mild (100|–300 IU/ml); moderate (300|–1000 IU/ml), and severe (> 1000 IU/ml). Values > 2000 IU/ml were considered over-flow $*p = 0.02$.

DISCUSSION

The taeniosis-cysticercosis complex occurs mainly in less developed countries. *T. solium* is widespread through South America, India, and China, and may also be endemic in sub-Saharan Africa (Pal et al. 2000). *T. saginata* is the main cestoda found in Europe with 0.01–10% of prevalence, although it can reach a prevalence of 2–10% in Afghanistan, Azerbaijan, India, and Malaysia (Cabaret et al. 2002). It has a moderate prevalence in South American countries and 0.01% in Brazil. Nonetheless, the knowledge of the infection in cattle or humans is insufficient, due to the lack or inexactness of information (Sakai et al. 2001, Cabaret et al. 2002, Agapejev 2003).

In this study we found a frequency of 5.9% of anti-*T. solium* cysticercus antibodies detected by ELISA screening assay, carried out through a 18–14 kDa purified antigenic protein from VF-Tcr. The reactive ELISA samples were tested by immunoblotting as a confirmatory test, and 3.6% resulted immunoreactive to the immunodominant 18- and 14 kDa peptides. Individuals with specific anti-*T. solium* cysticercus antibodies were adults. Two of them

came from Pontal of Paranapanema region and one from the northern of Paraná, an endemic region for cysticercosis (Lonardoni et al. 1996). It has been shown that low-molecular mass peptides (≤ 20 kDa) found in *T. crassiceps* are similar to *T. solium* antigens extracts, and considered specific for cysticercosis, improving the specificity of screening and confirmatory tests, especially in serum samples which cross-react with other cestoda antigens (Bueno et al. 2000, Pardini et al. 2002). ELISA tests showed sensibility of 100% in patients diagnosed with NC in our survey. The frequency of anti-*T. solium* cysticercus antibodies in the studied population is higher than those found by Bragazza et al. (2002), using a similar methodology in a population of Cássia dos Coqueiros (2.1%), a rural area near Ribeirão Preto, São Paulo, also considered an endemic region for cysticercosis. Surveys by ELISA by Vaz et al. (1990) in a population from five counties of São Paulo, and Silveira-Lacerda et al. (2002) in blood donors from four cities in Minas Gerais found a frequency of anti-*T. solium* cysticercus antibodies of 2.3 and 5.6% respectively. Using indirect immunofluorescence assay, Lonardoni et al. (1996), in individuals of five counties from northern of Paraná, found a frequency of 3.2%. However, in the studies reported above, the results were not confirmed by immunoblot, and some of the reactive samples could be false-positive results, since screening methods lack specificity (Tsang et al. 1989). In a recent literature review, seroepidemiological surveys in the South and Southeast region of Brazil, including São Paulo, showed 0.8% cysticercosis positivity for children, and 2.3% positivity in the general population (Agapejev 2003).

Before land expropriation and distribution, most individuals of our studied population lived 2–3 years in a marginal land camping organized by the “Landless Peasants’ Movement”. As already mentioned, these campings are characterized by poor living conditions, deficient potable water supply and sanitary facilities, and could be a focus of taeniosis infection. Socioeconomic status is shown in scientific literature as being one of the key conditions to acquire most of the infections in parasitic diseases, including taeniosis and cysticercosis, especially in the tropical and subtropical world.

In animal models and clinical surveys, helminthic infections, such as taeniosis, are associated with eosinophilia and high levels of IgE, once they act together in the immune response against parasites (Gounni et al. 1994). Rodriguez-Sosa et al. (2004) demonstrated that mice infected with *T. crassiceps* displayed high levels of IgE and a Th2 immune response. Total and specific IgE were also found in serum samples and cerebrospinal fluid of patients with cysticercosis (Short et al. 1990, Bueno et al. 2000). Furthermore, Chavarria et al. (2005) showed increased levels of eosinophils in cerebrospinal fluid of patients with cysticercosis. Indeed, all of the individuals in our survey which resulted positive for anti-*T. solium* cysticercus antibodies showed elevated levels of IgE, although they were not correlated with eosinophilia. In the whole population studied, 93.9% of individuals presented an increase in total IgE concentration and a positive correlation with increased eosinophils counts (mild and moderate levels) was found ($*p = 0.02$). These data suggest

that other antigens than helminthes infection are triggering this immune response.

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 and elsewhere (Kobayashi et al. 1995, Carvalho et al. 2002, Abd El Bagi et al. 2004). In a survey among "Landless Peasants Communities" in Rio Grande do Sul, De Carli et al. (1994) showed intestinal parasites infection prevalence of 42.4% in Charqueadas and 51% in Capela Santana, being that in the latter, 1.3% of individuals presented eggs of *Taenia* sp. too. The low prevalence of parasites in our studied population may be due to the medical assistance and treatment received by the community by the Family Health Program, with weekly medical visit to the settled Pe. Josimo Peasants Settlement (pers. commun.).

We conclude that the detection of anti-*T. solium* cysticercus antibodies in individuals of Pe. Josimo peasants' settlement is higher than other regions considered endemic in São Paulo. Besides, the increased levels of IgE and eosinophils could be an immune response to environmental antigens and should be investigated. Thus, it is important to carry out surveys in peasants' settlement areas with the objective of establishing public health measures for prevention and control of infectious diseases such as taeniosis-cysticercosis.

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