

BRIEF COMMUNICATION

ANTI-*Taenia solium* METACESTODES ANTIBODIES IN SERUM FROM BLOOD DONORS FROM FOUR CITIES OF TRIÂNGULO MINEIRO AREA, MINAS GERAIS, BRAZIL, 1995

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SUMMARY

Serological survey was performed to detect IgG antibodies anti-*Taenia solium* metacestodes in blood donors of Hemocentro Regional de Uberlândia, Minas Gerais, Brazil. A total of 1133 sera from blood donors coming from four cities of Triângulo Mineiro area were analyzed by the indirect fluorescence antibody test (IFAT) and the enzyme linked immunosorbent assay (ELISA). Specific IgG antibodies were found in 5.6% of the studied population, showing differences in the positive rates according to their origin: Araguari (13.5%), Tupaciguara (5.0%), Monte Alegre de Minas (4.8%) and Uberlândia (4.7%). The results indicate the probable endemicity of cysticercosis in this population.

KEYWORDS: Human cysticercosis; Blood donors; Seropositivity.

Taeniasis cysticercosis complex represent a serious problem for the public health in many parts of the world²⁸ including Brazil^{14,27}. In the areas where the infection is endemic, the transmission is clearly related to the poor personal hygiene and deficient environmental sanitation, specially in rural areas where swine are created in rustic manage conditions²⁰.

It has been estimated by World Health Organization that 50 millions subjects are infected by the taeniasis/cysticercosis complex and 50,000 of them die in every year⁵.

In Brazil, there are various studies on the frequency of cysticercosis in the human population. The majority of the reported cases are related to disease-cases restricted to the neurology and neurosurgery specialized centers or to anatomo-pathological materials coming from psychiatric and common hospitals, which show a high frequency in the states of Paraná¹⁴, São Paulo^{3,26}, Minas Gerais^{8,11,23} and Rio Janeiro¹⁸. However, the population prevalence is not known because of the absence of notification of the disease²⁴.

The aim of this study was to determine the seropositivity of IgG antibodies anti-*Taenia solium* metacestodes in blood donors of Hemocentro Regional de Uberlândia. Uberlândia is enclosed in a micro-region of the Triângulo Mineiro with 500,095 habitants, receiving immigrants from all adjacent cities since it is the convergence center of the entire Triângulo Mineiro.

This study received approval from the Ethical Committee of Fundação Hemominas, MG.

Serum samples were obtained from blood donors, during May and June 1995, and stored at -20 °C. Sampling was calculated by using the Rodrigues' technique¹⁹, according to the formula: $n = Z^2 \times P \times Q/d^2$. The 99% confidence level and 1.4% observed value were considered as described by COSTA-CRUZ *et al.*⁸. Thus, the sampling size was 660, being augmented to 1133 sera obtained from subjects coming from four cities of Triângulo Mineiro area: Uberlândia (888), Tupaciguara (120), Araguari (104) and Monte Alegre de Minas (21).

Serological assays included the indirect immunofluorescence test (IFAT) by using particulate antigen of *Cysticercus cellulosae*¹⁵ and enzyme linked immunosorbent assay (ELISA) by using total saline extract of *C. cellulosae*⁷ to detect IgG antibodies anti-*T. solium* metacestodes. The quantitative ELISA⁶ with modification was carried out using polystyrene microplates (Interlab, São Paulo, Brazil) and the reagents were assayed in 50 µL/well. The plates were coated with saline extract at 10 µg/mL in 0.06M carbonate-bicarbonate buffer, pH 9.6 and incubated overnight at 4 °C. The plates were washed three times for 5 minutes with PBS containing 0.05% Tween 20 (PBS-T) and incubated with the serum samples, including three positive and three negative control sera, diluted at 1:200 in PBS-T for 45 minutes at 37 °C. After new washing as previously described, the conjugate rabbit anti-human IgG (Fc chain specific) labeled with peroxidase (Sigma, USA) diluted

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Table 1

Frequency of IgG antibodies anti-*Taenia solium* metacestodes in blood donors of Hemocentro Regional of Uberlândia, according to sex and their origin

Cities	Male		Female		Total	
	No. of samples	No. of reactive samples (%)	No. of samples	No. of reactive samples (%)	No. of samples	No. of reactive samples
Uberlândia	678	28 (4.1)	210	14 (6.7)	888	42 (4.7)
Araguari	80	14 (17.5)	24	0 (0.0)	104	14 (13.5)
Tupaciguara	71	5 (7.0)	49	1 (2.0)	120	6 (5.0)
M. Alegre	12	0 (0.0)	9	1 (11.1)	21	1 (4.8)
Total	841	47 (5.6)	292	16 (5.5)	1133	63 (5.6)

at 1:2000 in PBS-T was added and incubated for 45 minutes at 37 °C. After washing, the enzymatic substrate consisting of H₂O₂ (Merck, Germany) plus o-phenylenediamine (OPD) diluted in 0.1M citrate-Na₂HPO₄ buffer pH 5.5 was added. The reaction was stopped after 15 minutes with 20 µL/well of 1M H₂SO₄ and the absorbance values were determined in an ELISA reader (Metrolab, Argentina) at 490 nm. The cut off was set using the mean absorbance values obtained from 3 non-reactive sera plus two standard deviations. Serum samples were considered as reactive when presenting antibody titers in both IFAT (≥ 20) and ELISA (≥ 200) as well as when presenting antibody titers in either IFAT (≥ 40) or ELISA (≥ 400).

From 1133 studied subjects, 74.2% were male and 25.8% female, and the age ranged from 18 to 60 years. IgG antibodies anti-*T. solium* metacestodes were found in 5.6% of the studied population, and the distribution of the frequencies of positivity according to their origin is demonstrated in Table 1. The mean age of the seropositive subjects was 33.5 years for male and 47.5 years for female. The general distribution of 1133 serum samples according to the antibody titers in IFAT and ELISA is demonstrated in Table 2.

Considering ELISA as reference, the tests showed 1070/1082 (98.9%) co-negativity, 24/51 (47.0%) co-positivity and 96.6% concordant results.

The mean rate of 5.6% found for antibodies anti-*T. solium* metacestodes in sera from blood donors of the four cities of Triângulo Mineiro area indicate the probable endemic character of cysticercosis in the region, since the Pan-American Health Organization¹⁶ sets the index of 0.1% as endemic for human cysticercosis. Necropsy data of subjects from this region demonstrated the mentioned endemic index^{8,11,13}.

Table 2

Distribution of the 1133 serum from blood donors according to the antibody titers found in the serological *Taenia solium* metacestodes tests (IFAT and ELISA)

IFAT(Titers)	ELISA (Titers)				
	< 200	200	400	800	1600
<20	979	35	18	08	01
20	56	12	03	0	0
40	10	04	01	0	0
80	02	01	01	02	0

AGAPEJEV² stated that the lethality caused by neurocysticercosis (NC) was 2.2%. Undoubtedly, NC is the responsible for the most of the obits, either by severity of the disease or by resulting of complications in the treatment.

In 1996, diagnostic criteria for cysticercosis, based on the objective evaluation of clinical, radiologic, immunologic and epidemiologic data were proposed⁹ and in 2001¹⁰ a panel of experts agreed upon more accurate and stringent revised criteria for diagnosis of NC. Furthermore, serology of NC may be plagued with apparently false positive results for several reasons: some healthy subjects from endemic countries may have serum antibodies induced by previous infections that did not progress to the establishment of cysticerci, or because they bear cysticerci that are localized in clinically inconspicuous anatomic sites²¹. Also, a major cause of truly false positive serology in methods using whole or partially purified antigen preparations is the extensive sharing of antigen epitopes of many cestodes and helminths¹².

ELISA standardized for crude antigens has been used in the diagnosis of human cysticercosis^{3,4,6,22} but due the frequency of cross-reaction observed in endemic areas, as well as to other helminthic infections, differences of sensibility and specificity have been found. In this work, the 1:200 dilution used in ELISA decrease cross-reaction indices in sera²². Despite the high specificity of the enzyme-linked immunoelectrotransfer blot assay (EITB) and glycoprotein antigens for diagnosing human cysticercosis²⁵, cross reaction with other parasites may occur and the wide divergence in sensitivity of serological tests reflects differences not only in the methodology employed, but also in the characteristics of the population investigated³. ELISA and EITB together may supply reliable results for the diagnosis of human cysticercosis^{3,22}, but EITB is too costly for application to populations of developing countries. Reports of the literature have reinforced the necessity in developing more reliable immunological methods to detect antibodies anti-metacestodes in serum or cerebrospinal fluid^{1,4,7,16,17}.

According to OPAS¹⁶, the compulsory notification of cysticercosis is the best resource for the epidemiological investigation and comparative analysis of prevalence in the different locals. In addition, such notification permits the mapping of the local of patients' homes in order to better guide the prevention measures, however, although compulsory, the notification of cysticercosis is still underestimated. The results obtained in this work indicate the probable endemicity of cysticercosis in this population and aimed to contribute for a better knowledge of the infection in our region, alerting for the fact that control and prevention measures

should be taken at primary level of assistance to health in the public services.

RESUMO

Anticorpos anti-formas metacestódeas de *Taenia solium* em amostras de soros de doadores de sangue de quatro cidades da região do Triângulo Mineiro, Minas Gerais, Brasil, 1995

Realizou-se pesquisa sorológica para detectar anticorpos IgG anti-formas metacestódeas de *Taenia solium* em doadores de sangue do Hemocentro Regional de Uberlândia, Minas Gerais, Brasil. O total de 1133 amostras de soros de doadores de sangue de quatro cidades do Triângulo Mineiro foi analisado pelo teste de imunofluorescência indireta (IFI) e o teste imunoenzimático (ELISA). Anticorpos IgG específicos foram detectados em 5,6% da população estudada, mostrando diferenças nas taxas de positividade de acordo com suas cidades de origens: Araguari (13,5%), Tupaciguara (5,0%), Monte Alegre de Minas (4,8%) e Uberlândia (4,7%). Os resultados indicam a provável endemicidade de cisticercose nesta população.

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