

BRIEF COMMUNICATION

ANTI-*Taenia solium* METACESTODE IgG ANTIBODIES IN SERUM SAMPLES FROM INHABITANTS OF A CENTRAL-WESTERN REGION OF BRAZIL

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SUMMARY

A total of 354 serum samples from inhabitants who frequent the Clinical Laboratory in Catalão, Goiás, in the central-western region of Brazil, were collected from June to August, 2002. The samples were evaluated by indirect immunofluorescence antibody tests and an enzyme linked immunosorbent assay in order to detect anti-*Taenia solium* metacestode IgG antibodies. Reactive and inconclusive samples were tested by Western blotting (WB). Considering WB as a confirmation, the frequency of antibodies in the serum samples of the above population was 11.3% (CI 5.09 – 17.51). The immunodominant bands most frequently recognized in WB were 64-68 kDa (97.5%) and 47-52 kDa (80%). The percentage of seropositivity to cysticercosis was significantly higher for individuals residing in areas without sewage systems ($p < 0.0001$). In conclusion, the results indicate a probable endemic situation of cysticercosis in this population. These results reinforce the urgent need for control and prevention measures to be taken by the local public health services.

KEYWORDS: *Taenia solium*; Cysticercosis; Immunodiagnosis; Brazil.

Human cysticercosis, caused by the ingestion of *Taenia solium* eggs, represents a public health concern in developing as well as developed countries where immigration is a major risk factor^{11,13,15}. Human neurocysticercosis is a major cause of epileptic seizures and other neurological morbidity worldwide^{2,8}. The true impact of this disease; however, has been obscured by the lack of sensitive and specific diagnostic tools for epidemiological data⁵.

The indirect immunofluorescence antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA) have been proved to be adequate for serum screening in cysticercosis studies^{3,6,17}. Using these two methods, VIANNA *et al.*¹⁹ in a seroepidemiological study of the human population in various geographical regions of Brazil estimated the frequency of cysticercosis seroprevalence at 8.11% in the South-east region, 5.81% in the North-east, 5.3% in the Central West and 3.5% in the Southern part of the country. The use of Western blotting (WB) represents a significant advance because it allows the identification of specific antigenic proteins and eliminates false-positive results that are common in the use of the IFAT and ELISA tests^{2,8,16}.

The occurrence of *T. solium* metacestode infection is unknown among the inhabitants of Catalão, Goiás, in the central-western region of Brazil. Thus, the aim of this study was to identify any possible

existence of *T. solium* metacestode IgG antibodies in serum samples from these inhabitants using IFAT, ELISA and WB.

The municipality (including both the urban and rural areas) of Catalão (18° 10' 05" S and 47° 57' 19" W) is 300 km from Brasília, the capital of Brazil, in the central-western region. The area is 3,789.5 km², with a population of 64,347 inhabitants of which the majority (89.5%) lives in the urban area. Homes with indoor plumbing account for 96%, however only 49% are connected to sewage system facilities.

Informed consent was obtained from all human adult participants or legal guardians of minors together with the approved consent of the Ethical Committee of the Universidade Federal de Uberlândia, Minas Gerais, Brazil.

A total of 354 serum samples were collected from individuals who attended the Clinical Laboratory in Catalão from June to August 2002. Sample calculations were elaborated following the methods of RODRIGUES¹⁴. The samples were analyzed in order to detect the seroprevalence of cysticercosis. Blood samples from the 354 subjects were obtained by venipuncture of the arm. Sera were stored at -20 °C until the time of examination in the Parasitology Laboratory of the Universidade Federal de Uberlândia.

All participants answered a questionnaire informing their demographic characteristics (gender, age), hygienic habits and sanitary conditions.

Preliminary experiments were carried out in order to determine the optimal conditions for IFAT, ELISA and WB, through antigen, sera and conjugate block titration.

IFAT, using *T. solium* metacestode antigen, was conducted according to MACHADO *et al.*¹⁰. The serum samples were diluted at 1:20 and the fluoresceinated anti-human IgG conjugate (Salk, Brazil) was added at 1:60. The slides were examined in a Zeiss immunofluorescence microscope (x 200 and 400). Results were expressed in titers, and considered positive at levels of ≥ 20 . Positive samples were retested using serial dilutions, for antibody titration.

ELISA was performed as described by SHIGUEKAWA *et al.*¹⁶ using polystyrene microplates (Interlab, São Paulo, Brazil). The plates were coated with the total saline extract antigen from 50 metacestodes of *T. solium* (50 μ L/well), obtained according to COSTA *et al.*⁴, at a concentration of 10 μ g/mL. The serum samples were diluted at 1:200 and the conjugate, goat anti-human IgG (Fc chain specific) labeled with peroxidase (Sigma, St. Louis, USA), was used at a titre of 2000. The reaction was developed by adding enzyme substrate consisting of 0.03% H₂O₂ (Merck, Germany) in 0.1 M citrate -Na₂HPO₄ buffer (pH 5.0) containing 0.4 mg/mL of o-phenylenediamine (Merck). After 15 minutes at room temperature the reaction was stopped by adding 1 M H₂SO₄ (25 μ L/well) and the absorbance values were determined in an ELISA reader (Titertek Multiskan Plus, Flow Laboratories, USA) at 492 nm. The cut-off point was set using the mean of three non-reactive samples plus two standard deviations. Results were expressed in titers which were considered positive at ≥ 200 . Positive samples were retested using serial dilutions, for antibody titration.

The reactive and inconclusive (one positive test was observed) samples were tested by WB. The total saline extract antigen was submitted to electrophoresis in sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) at 12% according to LAEMMLI⁹. After the SDS-PAGE process, the gels were transferred to nitrocellulose membranes (0.45 μ m; Sigma) as described by TOWBIN *et al.*¹⁸ using a semidry transfer apparatus (Multiphor II, Pharmacia-LKB). The WB assay was performed according to SHIGUEKAWA *et al.*¹⁶ with serum samples diluted at 1:50 and anti-human IgG (whole molecule) peroxidase conjugate at a titre of 150. At least two or more immunodominant proteins (24, 39-42, 47-52, 56, 64-68, 126-155 kDa), recognized by the sera sample, were considered seropositive.

Comparisons between the cysticercosis seropositive individuals and the epidemiological factors were made using Fisher's exact test. Results were considered significant at $p < 0.05$ and 95% confidence intervals (CI).

Considering the WB as a confirmation test, the prevalence of anti-*T. solium* metacestode IgG antibodies in the serum samples of the studied population was 40/354 (11.3%; CI 5.09 - 17.51). There were positive, concurrent results among all three tests in 24 serum samples. Another 16 samples demonstrated consistency between WB reagents and IFAT (13 cases) and with ELISA (three cases). The titers of the

reactive serum samples varied from 20 to 160 for IFAT and from 200 to 400 for ELISA. In each WB reactive serum sample two or three immunodominant proteins were recognized. Overall, the immunodominant bands most frequently recognized were those of 64-68 kDa (97.5%) and 47-52 kDa (80%), followed by the 56 kDa (40%), 39-42 kDa (30%), 24 kDa (5%). The 126-155 kDa band was not recognized.

In Brazil, the majority of the reported cases of anti-*T. solium* metacestode IgG antibodies are related to cases of disease being treated

Table 1

Association between cysticercosis' seropositivity (IFAT, ELISA and WB) and demographic and epidemiological characteristics of 354 inhabitants of one municipality in the central-western region of Brazil, from June to August 2002

Characteristic	n	Reactive (%)	95% CI
Gender			
Male	169	12 (7.1)*	2.10-12.10
Female	185	28 (15.0)*	8.00-22.00
Age (years)			
≤ 15	23	6 (26.1)	17.48-34.70
16 – 35	157	15 (9.6)	3.79-15.31
36 – 55	140	14 (10.0)	4.12-15.88
≥ 56	34	5 (14.7)	7.77-21.65
Familiar income (US\$)			
≤ 130.00	34	5 (14.7)	7.77-21.65
130.00-390.00	157	15 (9.6)	3.79-15.31
390.00-650.00	140	14 (10.0)	4.12-15.88
≥ 650.00	23	6 (26.1)	17.48-34.70
Indoor plumbing			
Yes	347	39 (11.2)	5.02-17.38
No	7	1 (14.3)	7.44-21.16
Contact with swine			
Yes	106	12 (11.3)	5.11-17.53
No	248	28 (11.3)	5.10-17.50
Consumption of raw meat			
Yes	14	2 (14.3)	7.44-21.16
No	340	38 (11.2)	5.02-17.38
Sewage system			
Yes	252	9 (3.6)**	0.00-7.20
No	102	31 (30.4)**	21.38-39.42

* $p = 0.0188$; ** $p < 0.0001$; CI: Confidence interval

in neurological and neurosurgical specialized centers or to anatomopathological materials coming from psychiatric and public hospitals. Levels of prevalence are not known because of the absence of notification of the disease¹⁷. The prevalence of anti-*T. solium* metacestode IgG antibodies in sera from the inhabitants of the study area indicates a probable endemic character of cysticercosis in the region since the Pan American Health Organization¹² sets the index of 0.1% as endemic for human cysticercosis. Previous results¹⁹ demonstrated only 5.3% of seroprevalence using IFAT and ELISA in the central-western region of Brazil. However, this study was carried out in Brasília-DF, 350 Km from Catalão. In a serological survey performed in blood donors from Araguari, city of Triângulo Mineiro area, 70 Km from Catalão, SILVEIRA-LACERDA *et al.*¹⁷ detected specific IgG antibodies in 13.5% of the studied population. The proximity between the cities with the same socio-economic and sanitary characteristics permitting the identification of geographic niches of the disease, with the consequent creation of satisfactory mechanism of intervention reducing the impact of the disease on public health.

The association between seropositivity and epidemiological factors for cysticercosis can be observed in Table 1. The seroprevalence was significantly higher in females ($p = 0.0188$). The population studied range in age from six to 81 years with the adults' serum samples being more reactive. The results agreed with other studies^{1,3,5}, indicating that the higher prevalence among adults was presumably due to their longer period of exposure and/or different immune response¹⁵.

Of the total of 354 individuals, 252 (71.2%) are connected to sewage systems. In fact, the percentage of seropositivity to cysticercosis was statistically higher among individuals lacking a link to a sewage system ($p < 0.0001$). These results showed the importance of improving sanitary practices at the household level to reduce the prevalence of the disease, as stated by FLISSER *et al.*⁷. According to VIANNA *et al.*¹⁹ in Brazil the absence of sanitary conditions in the home, close contact with swine, and the domestic use of river water constituted factors of risk, with the respective values of 3.1, 2.2 and 1.8.

In conclusion, the results indicate a probable endemic situation of cysticercosis in the population examined. These results reinforce the urgent need for control and prevention measures to be taken by the local public health services.

RESUMO

Anticorpos IgG anti-metacésteo de *Taenia solium* em amostras de soro de habitantes da região centro-oeste do Brasil

Um total de 354 amostras de soro de habitantes que freqüentaram o Laboratório Clínico em Catalão, Goiás, na região centro-oeste do Brasil, foram colhidas no período de junho a agosto de 2002. As amostras foram avaliadas pelo teste de imunofluorescência indireta e *enzyme-linked immunosorbent assay* (ELISA) com o objetivo de detectar anticorpos IgG anti-metacésteo de *Taenia solium*. As amostras reativas e inconclusivas foram testadas pelo *Western blotting* (WB). Considerando WB como reação confirmatória, a freqüência de anticorpos nas amostras de soro da população estudada foi 11,3% (IC: 5,09 - 17,51). As bandas imunodominantes mais frequentemente reconhecidas no WB foram 64-68 kDa (97,5%) e 47-52 kDa (80%). A porcentagem de soropositividade

para cisticercose foi significativamente maior nos indivíduos que residiam em áreas sem sistema de esgoto ($p < 0,0001$). Concluiu-se que os resultados indicam uma provável situação de endemidade para cisticercose nesta população, reforçando a urgente necessidade de medidas de controle e prevenção que devem ser implantadas pelo serviço de saúde pública local.

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