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Original Article

Seropositivity to Cysticercosis in School-Age Children Living in a Low-Income Municipality in the Midwest Region of Brazil

Dayane Moraes¹, Émelin Alves Dos Santos¹, José Antônio Alves Mendes¹,
Ivanildes Solange Da Costa Barcelos², João Batista Alves De Souza¹, Maria Do Rosário De Fátima
Gonçalves-Pires³, Julia Maria Costa-Cruz³, *Rosângela Maria Rodrigues¹

1. Parasitology Laboratory, Health Sciences Academic Unit, Federal University of Jataí, Goiás State, Brazil
2. Immunology Laboratory, Health Sciences Academic Unit, Federal University of Jataí, Goiás State, Brazil
3. Parasitic Diagnosis Laboratory, Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, Minas Gerais State, Brazil

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*Correspondence Email:

rosangela_rodrigues@ufj.edu.br

Abstract

Background: Human cysticercosis (CC) is a global public health problem, especially in Latin America, including Brazil. We aimed to analyze the seroprevalence of CC among school-age children and adolescents.

Methods: We analyzed the presence of specific IgG antibodies against *Taenia solium* metacestodes in 500 serum samples from elementary school children and adolescents in Jataí City, state of Goiás, Brazil. IgG antibodies against the antigenic extract of the parasite were detected and analyzed by ELISA, and specific peptides were identified by confirmatory Western Blotting test.

Results: Of the 500 study participants, 205 (41%) were male, and 295 (59%) were female. Participants aged between 4 and 18 years (mean age 8.4 years). The percentage of serum samples reactive by ELISA was 37.2%. These samples were analyzed by Western Blotting, which confirmed that the seropositivity rate was 6.2% (95% CI 2.4–14.7) in 31 samples reactive for CC-specific bands, determined in serum samples from 18 male (5–11 years old) and 13 female (4–12 years old) students.

Conclusion: The CC seroprevalence demonstrated in schoolchildren suggests that this parasitosis is endemic in the study area. Further investigations are necessary to clarify the local epidemiology of this parasitosis.



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Introduction

Human cysticercosis (CC) is a neglected and zoonotic helminthic disease acquired through the ingestion of *Taenia solium* eggs, which release infective embryos that enter host tissues and encyst to form larvae metacestodes (1). Its infection route is associated with poor food hygiene, poor drinking water quality, and poverty (1, 2). The parasite can infect organs and tissues, especially skeletal muscles and eyes, not only in humans but also in pigs, which are natural intermediate hosts (2). Neurocysticercosis (NCC) is the most harmful clinical form of this parasite in humans and can cause severe neurological conditions such as epilepsy, neurological deficits, increased intracranial pressure, and cognitive decline (3). NCC is an important cause of epilepsy, psychomotor involution, behavioral changes, and learning difficulties in children and adults (4-6).

CC occurs worldwide and is highly prevalent in Latin America, Asia, and sub-Saharan Africa (1). CC has an endemic occurrence in Brazil, mainly in the Southeast, South, and Midwest regions. It caused 2,007 deaths in most Brazilian states from 2000–2011 (7). In the southeastern Brazilian region, IgG antibodies against CC were detected in 13.5%, 5.0%, 4.8%, and 4.7% of blood donors in the cities of Araguari, Tupaciguara, Monte Alegre de Minas, and Uberlândia, Minas Gerais (8). In another study, a prevalence of 5.7% was reported for IgG antibody detection in a rural settlement in São Paulo (9). In the southern region, CC seroprevalence was 3.4% in Lages-SC (10). In the Midwest, a study demonstrated a seroprevalence of 13.8% for CC in adults living in the municipality of Jataí-GO (11).

Despite the high prevalence rates in Brazil and the aggressiveness of the cases registered for this disease worldwide, epidemiological screening studies in the country do not mention the infection rates in children and young adults. In this sense, serological and epidemiological analyses of the prevalence of CC in

children are essential for monitoring the number of cases and adopting appropriate treatment and control measures, including education on good personal and food hygiene practices (2, 5, 12). We aimed to analyze the seroprevalence of CC among school-age children and adolescents.

Methods

We analyzed the seroprevalence of IgG antibodies against CC in the serum samples of 500 school-age students. The study was conducted in four public schools in the city of Jataí-GO (17° 52' 53" S and 51° 42' 52" W), selected with the consent of the municipality secretary of education. Jataí City is located in southwestern Goiás State, Midwestern Brazil, approximately 330 km from Goiânia City (capital). It has a total area of 7,174 km² and an estimated population of 103,221 inhabitants (13).

In these institutions, parents or custodians were introduced to the project and invited, upon free demand, to allow children to participate in the study. Only students whose parents or custodians signed the Terms of Free and Informed Consent form were included in the analyses.

Blood samples were collected from 500 students by venous puncture, and the sera were separated, identified, and stored at -20 °C until use. The Research Ethics Committee of the Federal University of Goiás (UFG) approved the study protocol (number 335/2010). The parents or guardians of all participants signed an informed consent form to authorize blood collection and analysis.

The tests were performed using a total saline extract prepared with 50 *T. solium* metacestodes, and the protein concentration was 8,100 µg/mL (14, 15). All the sera samples were diluted to 1:100 in 0.05% Tween 20 solution in 0.01 M phosphate buffered saline pH 7.2 (PBS-T) and analyzed using enzyme-linked immunosorbent assay (ELISA) (16). Optical density (OD) was recorded at 490 nm using a microplate reader (Thermo plate TP-Reader, China). The cut-off value was calculated for each plate from the mean OD of nine nonreactive sera for CC (controls) plus three standard deviations. The OD sample/OD cut-off ratio was expressed

as the ELISA index (EI), and EI > 1.0 was considered positive.

Western blotting (WB) was used as a confirmatory test based on the recognition of specific peptides with 70, 64–68, 47–52, 39–42, 28–32, 24, and 18 kDa present in the *T. solium* larvae saline extract (16, 17). A serum sample was considered reactive WB when at least two of the seven peptides specific for CC were recognized (12, 13).

The seroprevalence of CC was statistically analyzed using test *t* de student, chi-square, and Fisher’s exact tests using GraphPad Prism

software (version 5.0). Statistical significance was set at $P < 0.05$.

Results

Of the 500 school-age children and adolescents who participated in the study, 205 (41%) were male, and 295 (59%) were female. Participants aged between 4 and 18 years (mean age of 8.4 years). Serum samples reactive according to ELISA and WB were 37.2% and 6.2%, respectively. There were no statistically significant differences in sex, age range, or positivity rate ($P = 0.4151$) by ELISA (Table 1).

Table 1: Distribution of the 500 serum samples from schoolchildren analysed by ELISA for the detection of anti *T. solium* metacestodes IgG, from February 2011 to November 2014, in the municipality of Jataí, Goiás, Brazil

Age group (yr)	Total participants			Positivity by ELISA		
	Male	Female	N	Male	Female	N (%)
4 – 6	64	70	134	25	29	54 (40.3)
7 – 9	69	125	194	27	48	75 (38.6)
10 – 11	48	62	110	15	16	31 (28.2)
12 – 14	19	33	52	6	16	22 (42.3)
≥ 15	5	5	10	2	2	4 (40.0)
Total	205	295	500	75	111	186 (37.2)

The WB analysis of 186 serum sample reagents by ELISA detected 31 samples reactive for CC-specific bands, 57 samples reactive for nonspecific bands (> 70 kDa), and 98 nonreactive samples. The 31 serum samples that were positive on both ELISA and WB represented 6.2% (95% CI 2.4–14.7) of the total CC seroprevalence (31/500). Eighteen and 13 of the 31 serum samples were collected from male (5–11 years old) and female students (4–12 years old) students, respectively.

The IgG antibodies present in the 31 serum samples recognized bands of 70, 64–68, and 47–52 kDa in the antigenic extract, with frequencies of 77.4%, 74.2%, and 64.5%, respectively (Table 2). The 24 kDa band was recognized in 44.4% of the 18 serum samples from male students; this recognition rate significantly differed from the recognition rates of the 39–42, 28–32, and 18 kDa bands ($P = 0.0178$).

Table 2: Recognition frequency of specific peptides for cysticercosis in 31 serum samples from schoolchildren reactive by ELISA and Western Blotting test

Specific peptides in the total saline extract (kDa)*	Recognition Frequency		
	Total (31) N (%)	Female (13) N (%)	Male (18) N (%)
70	24 (77.4)**	9 (69.2)	15 (83.3)
64-68	23 (74.2)**	10 (76.9)	13 (72.2)
47-52	20 (64.5)**	11 (84.6)	9 (50.0)
39-42	3 (9.7)	2 (15.4)	1 (5.5)

28-32	3 (9.7)	1 (7.7)	2 (11.1)
24	12 (38.7)	4 (30.8)	8 (44.4) [#]
18	2 (6.4)	0 (0.0)	2 (11.1)

© According Barcelos et al. (2007). kDa: kilo Daltons. N: number. (%): frequency. $p < 0.05$: in both gender^(*), in male only^(#)

Discussion

The present study demonstrated a 6.2% seroprevalence of CC in students from Mid-western Brazil, which can be compared with data from serological tests and epidemiological aspects related to the endemicity of this parasitosis.

The ELISA assay reported here detected an elevated positivity rate of IgG antibodies to CC because of (i) the high sensitivity and low specificity of the method when using the total antigenic extract of *T. solium* Cysticercus from areas endemic for CC and other helminthic parasitosis and (ii) cross-reaction with antibodies against *Echinococcus granulosus*, *Enterobius vermicularis*, *Schistosoma mansoni*, *Strongyloides stercoralis*, *Taenia saginata*, and *Toxocara* spp. (2, 16, 17). The endemicity of some parasitic diseases was demonstrated in the state of Goiás (18, 19).

The main limitation of crude antigens is the occurrence of nonspecific reactions in the ELISA test, whereas the main limitation of serological tests is that positivity for IgG only indicates exposure to the parasite, but it does not necessarily confirm the occurrence of current infection (20). The enzyme-linked immunoelectrotransfer blot (EITB) assay using lentil lectin-purified glycoprotein extracts of parasites is the most reliable test for detecting specific antibodies to *T. solium* antigens in serum or cerebrospinal fluid (2, 20). However, EITB requires a complex antigen purification process for the test strips, which is both expensive and difficult to standardize, preventing its broad application (1).

Our research team previously reported a prevalence of 13.8% for CC in serum samples from individuals in clinical laboratories, including children (8.2%), adults (75.3%), and older adults (16.5%) of both sexes living in Jataí (11). In Catalão City, state of Goiás, a

seroprevalence of 11.3% for CC was significantly associated with the absence of an urban sewage network (22).

There are no reports on the prevalence of Pediatric CC and NCC in Brazil. However, an Indian study reported a prevalence of 37.7% for CC seropositivity in a sample of 61 children and adolescents (mean age, 15 years) with afebrile seizure treated at the teaching hospital in Andhra Pradesh Province; infected patients were predominantly male (23). The overall prevalence of IgG for CC detected by ELISA was 6% in fifth- and sixth-grade students from 27 schools in western Sichuan, China, with a mean age of 13 years (12). In Mali, 8% of the 805 serum samples from students aged 4–17 years were IgG-positive for the GST-rT24H *T. solium* antigen, as analyzed by the multiplex bead assay (24). In Madagascar, the prevalence of antigens detected by Ag-ELISA was 27.7% in the general population, with a high prevalence of active CC among schoolchildren in an urban setting (25).

In the present study, male children aged 5 and 11 years exhibited the highest positivity rates for CC in the WB confirmatory test. CC can affect both females and males, from children to older adults, with a peak incidence in the age range of 20–50 years (1, 2).

It is worth considering factors related to transmission and sanitary conditions that favour maintenance of the parasite's life cycle, including breeding pigs in the peridomicile, poverty, inadequate sewage treatment, and lack of drinking water for the local population (1, 12). These factors are also related to the 13.03% prevalence of taeniasis due to *T. solium* infection in the Latin American population, mainly in Brazil, Colombia, Ecuador, Guatemala, Honduras, Mexico, Peru, and Venezuela (26). The seropositivity for human taeniasis was 4.5% (26/577) in a village in northeastern Brazil (27). The true preva-

lence of NC in Brazil is not known because reporting cases is not compulsory in most of the country, except in the municipality of Ribeirão Preto in the state of São Paulo (28). Epidemiological data in the municipality of Jataí has shown that inadequate sewage treatment and sanitation, which was reported in 70.5% of households in the last demographic census, may contribute to the transmission of infection by *T. solium* eggs (29). Despite the lack of current data on the incidence of swine cysticercosis, the literature reports that the state of Goiás had approximately 2 million swine in 2020, and that there was 7.6% positivity for serum IgG against *T. solium* in 92 domestic swine raised in the city of Goiânia (30, 31). In addition, several cities in Goiás state have no sewage networks or access to treated water (32).

Conclusion

The seropositivity for CC in school-age children and adolescents detected in this study suggests that the study area is endemic for disease transmission. However, further investigations are necessary to clarify the details of the local epidemiology of this parasitosis.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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