



Article/Artigo

Seroepidemiological study of human cysticercosis with blood samples collected on filter paper, in Lages, State of Santa Catarina, Brazil, 2004-2005

Estudo soropidemiológico da cisticercose humana com amostras de sangue total coletado em papel filtro, em Lages, Estado de Santa Catarina, 2004-2005

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ABSTRACT

Introduction: Human serofrequency of antibodies against *Taenia solium* antigens was determined and risk factors for cysticercosis transmission were identified. **Methods:** Individuals (n=878) from periurban and rural locations of Lages, SC, were interviewed to gather demographic, sanitary and health information. Interviews and blood sample collections by finger prick on Whatman filter paper were performed from August 2004 to May 2005. Observation determined that 850 samples were suitable for analysis and were tested by ELISA using vesicular fluid of *Taenia crassiceps* heterologous antigen. To ensure the reliability of the results, 77 samples of the dried blood were matched with sera. The reactive samples were submitted to a serum confirmatory immunoblot (IB) test using purified *Taenia crassiceps* glycoproteins. **Results:** The ELISA results for the dried blood and serum samples were statistically consistent. ELISA was positive in 186 (21.9%) out of 850 individuals. A group of 213 individuals were asked to collect vein blood for IB (186 with positive result in ELISA and 27 with inappropriate whole blood samples) and 130 attended the request. The IB was positive in 29 (3.4%) out of 850 individuals. A significant correlation ($p = 0.0364$) was determined among individuals who tested positive in the IB assay who practiced both pig rearing and kitchen gardening. **Conclusions:** ELISA with dried blood eluted from filter paper was suitable for cysticercosis population surveys. In Lages, human infection was associated with pig rearing and kitchen gardening. The prevalence index was compatible with other Latin American endemic areas.

Keywords: Cysticercosis. *Taenia crassiceps*. Immunodiagnosis. Epidemiology.

RESUMO

Introdução: O primeiro levantamento sobre cisticercose humana e identificação dos fatores de risco associados à transmissão, foram realizados em Lages, SC. **Métodos:** Oitocentos e setenta e sete voluntários de regiões periurbana e rural foram entrevistados e forneceram informações demográficas e condições sanitárias e de saúde. Amostras de sangue foram coletadas por meio de punção digital em papel filtro entre agosto 2004 e maio 2005. Verificou-se que 850 amostras estavam adequadas para análise. No ELISA, utilizou-se o antígeno heterólogo líquido vesicular de *Taenia crassiceps*. Para assegurar a confiabilidade dos resultados de ELISA, foram pareadas 77 amostras de soro e sangue eluído do papel filtro. A confirmação do diagnóstico sorológico foi feita por *immunoblot* (IB) com glicoproteínas purificadas de *Taenia crassiceps*. **Resultados:** A reatividade de IgG eluída de sangue em papel filtro mostrou-se compatível com a dos soros correspondentes. A triagem por ELISA de 850 indivíduos revelou 186 (21,9%) positivos. De 213 pessoas convidadas a colher soro para IB (186 ELISA positivo e 27 com amostras de sangue total inadequadas), compareceram 130. O IB foi positivo em 29 (3,4%) de 850 amostras. Houve correlação significativa entre IB positivo e a prática de criação de suínos e de horta caseira ($p = 0,0364$). **Conclusões:** ELISA com sangue total em papel filtro mostrou-se adequado para inquéritos populacionais para cisticercose. A transmissão da cisticercose humana na área estudada mostrou correlação com criação suína doméstica e horta caseira. A prevalência obtida foi semelhante à relatada em áreas endêmicas da América Latina.

Palavras-chaves: Cisticercose. *Taenia crassiceps*. Imunodiagnóstico. Epidemiologia.

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INTRODUCTION

Serology is an appropriate screening technique for identifying possible carriers of specific parasitic infections. Although the first report of an immunodiagnosis method for cysticercosis was presented by the Brazilian researcher Arthur Moses in 1911¹, Brazil still lacks well defined human prevalence figures for cysticercosis, although the Ministry of Health made it compulsory to report cases after 1996².

Human cysticercosis is prevalent in many developing countries and is reemerging in societies where human migration is intense³. Despite the worldwide distribution of this parasite, the prevalence of neurocysticercosis (NC) is rarely higher than 10% and the number of symptomatic NC cases is even lower^{4,5}.

Difficulties related to acquiring expensive equipment for diagnosis, such as computed tomography (CT) scanners, inhibit current knowledge of the real prevalence of the disease in some Brazilian states⁶. Immunological assays for the detection of specific *Taenia solium* cysticercosis antibodies are a valuable tool when used together with brain imaging exams⁷⁻⁹. However, false-negative results can be obtained in the enzyme-linked immunosorbent assay (ELISA) with serum or cerebrospinal fluid (CSF) from proven NC patients, as well as false-positive results due to cross-reactivity with other parasites^{8,10}. The currently accepted gold standard antibody-based diagnostic assay was developed by the Centers for Disease Control and Prevention (CDC), Atlanta, USA^{11,12}. The enzyme-linked immunoelectrotransfer blot (EITB) test, which uses seven purified glycoproteins as antigens¹³, is highly sensitive and specific for the detection of active multiple lesions but, conversely, the sensitivity for detecting single and calcified lesions is low^{11,12,14}. All of the components of these diagnosis antigens have been previously characterized, expressed and cloned, eliminating the need for parasite material

as a source of antigens^{15,16}. Attempts to adapt these antigens to the ELISA rather than the EITB format, to make it less work intensive and suitable for use in large scale screening assays, have resulted in a considerable reduction in specificity¹⁷.

Some authors have demonstrated that larvae from *Taenia solium* and *Taenia crassiceps* (laboratory strain) share antigenic components^{18,19}, such as the 18 and 14kDa fractions from which have been considered specific for the immunodiagnosis of NC²⁰.

This paper reports the use of purified 18/14kDa proteins obtained by immunoaffinity chromatography, which provided good performance and high specificity for serum samples²¹.

Studies concerning neurocysticercosis have been conducted on epileptic patients^{10,22,23}. Ishida et al¹⁰ reported the first correlation between tomography exam results and a serological survey on epileptic neurocysticercosis patients conducted in the State of Santa Catarina, indicating the good features of ELISA and the immunoblot assay in discriminating active lesions from inactive (calcified) lesions.

In ELISA, blood serum is generally used to detect antibodies. Using microquantities of whole blood as specimens is more practical for large scale studies in seroepidemiological surveys with regard to preservation, storage and costs²⁴⁻²⁶.

The aims of this study were to assess the frequency of cysticercosis in a population from the State of Santa Catarina by ELISA, using *Taenia crassiceps* vesicular fluid as antigen and dried blood on filter paper as source of anti-cysticercus antibodies, in association with confirmatory immunoblot serum test, using 18-14kDa fractions from *Taenia crassiceps* cysticercus and establish an association between infection and sociodemographic and environmental factors.

METHODS

Study site

This study was conducted in the rural and periurban areas of Lages, a town in the State of Santa Catarina (SC), Brazil (27°48'S; 50°20'W) located around 200km southeast of the State capital Florianópolis, in southern Brazil. The main economic activity of the region is livestock production on small farms and other types of farming. The annual temperature varies from 7.4 to 35°C with an annual mean of 15°C. The subtropical climate has one rainy season from May to December and the average annual rainfall is 120mm. The total population of the municipality is 162,000.

This region has been identified as important in terms of cysticercosis²³ and, as with many other towns and cities in Brazil, it has inadequate sanitary and socioeconomic conditions that can facilitate the transmission of *Taenia solium* through open air defecation, fecal contamination of the environmental, rustic pig rearing methods, poor person hygiene and dietary habits and pork consumption without proper inspection by the authorities.

Study subjects

The population studied was a total of 877 individuals belonging to 580 families from eight locations in the rural area and 29 districts in the periurban area, located around the center of the town. In the periurban areas, the survey was conducted door to door by our staff, always accompanied by a community health worker from the Family Health Program of the Health Ministry. The rural inhabitants were interviewed at community health centers during their visits. All individuals in the study were interviewed using a questionnaire.

Demographic information concerning age, sex, education and occupation were collected, together with any history of teniasis/cysticercosis and personal hygiene habits. A family questionnaire was applied to each household with questions relating to pig-rearing, the origin of vegetables consumed, sanitation facilities in the home, among other information. The data were collected from August 2004 to May 2005.

Terms of free, informed consent were signed by all participant subjects or from parents or legal guardians of minors or individuals with mental illness. The exam results were sent to the Family Health Program Office. Individuals identified as immunoreactive for cysticercosis were advised to inform their local health service in a subsequent visit.

Samples

All 877 (594 females and 283 males) interviewees provided a blood sample collected on Whatman filter paper-3 (0.5cm x 1cm) by finger prick until each strip was completely soaked with blood on both sides. The Whatman filter paper samples were air dried at room temperature, labeled, sealed in airtight packets and stored at -20°C until use. For elution, the specimens were cut out and antibodies were eluted by soaking the samples in 240µl of PBS for 24h at 4°C. Since 27 samples were determined as inappropriate, these volunteers, along with those who were ELISA positive, were invited to attend to a health center to collect vein blood for a confirmatory immunoblot.

Of these, 130 individuals responded to the invitation and blood samples were obtained by vein puncture collected in sterile screw-capped bottles. The samples were allowed to clot at room temperature and the serum was then separated, labeled and stored in a deep freezer (-20°C) until testing (herein referred to as the classical method). A group of 77 individuals were tested for both sources of antibody by ELISA, serum and whole blood, in order to verify the agreement between the results.

Control samples: six serum samples from patients with NC confirmed by imaging exams (CT and/or MRI) and serological assays (ELISA and IB), obtained from the Immunology Laboratory of the Department of Clinical Analyses of São Paulo University (*Laboratório de Imunologia, Departamento de Análises Clínicas, Universidade de São Paulo*) serum collection, were used as a positive control group. Twenty six serum samples from blood donors collected from Hemotherapy Service of the University Hospital of the Federal University of Santa Catarina (*Universidade Federal de Santa Catarina, UFSC*), Brazil, were used as a negative control group.

All samples were examined at the Microbiology and Parasitology Department of the UFSC, Brazil.

Antigens

Cysts of the Ontario Research Foundation (ORF), Canada, strain of *Taenia crassiceps* were obtained from the peritoneal cavity of experimental infected Balb/C mice, as described by Vaz et al²⁷. Vesicular fluid of the *Taenia crassiceps* antigen (Tcra-vf) was produced according to the method described by Espíndola et al¹⁹. The 18-and 14-kDa proteins from *Taenia crassiceps* cysticerci (18/14-Tcra) were purified by immunoaffinity chromatography using a sepharose column coupled with monoclonal antibody anti-*Taenia crassiceps*, as described by Espíndola et al²¹.

Enzyme-linked immunosorbent assay

ELISA was performed with 850 total blood samples and 77 matched sera, according to previously standardized protocols with Tcra-vf¹⁸. Each well of the plates was coated with 10µg/ml of Tcra-vf antigen. The sera samples and whole blood were diluted 1:100

and 1:2.5, respectively, in a 1% skim milk saline solution containing 0.05% Tween 20. Conjugate peroxidase-labeled sheep anti-human immunoglobulin G was used at titers 1:5,000 for both sera and total blood sample and chromogen substrate ortho-phenylenediamine and H₂O₂ in citrate buffer (pH 5.0). The reaction was read with a plate spectrophotometer at 492nm. The cutoff value for the ELISA-Tcra-vf was determined with sera from 6 proven NC patients (positive controls) and from 68 healthy individuals (negative controls) following the same ELISA serum protocol¹⁸. Each plaque assay was performed with the inclusion of one positive and one negative serum sample as controls, from the same group of samples used in the standardization. The results were expressed as the ratio between the optical density (OD) of the sample and the cutoff value. Ratio values > 1 were considered as positive.

In order to compare the results obtained for the filter paper-eluted blood with those obtained for the serum samples, a dilution factor for the filter paper samples was defined based on the volume of blood soaked in the delimited paper area^{26,28}.

Immunoblot assay

The 18/14-Tcra antigen obtained by elution from the immunoaffinity chromatography with specific monoclonal antibodies was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)²⁹. Then (3µg/mm) was solubilized with sample buffer (0.01M Tris-HCl, pH 6.8, containing 2% SDS, 5% 2-mercaptoethanol, and 10% glycerol) at 100°C for 5min and separated electrophoretically on 15% polyacrylamide gel. The separated antigen was transferred to a 0.22-µm-pore size membrane of polyvinylidene difluoride (Millipore Corp., Bedford, Mass). The membrane was blocked by treatment with 5% skim milk in PBS for 2h, washed in PBS containing 0.05% Tween 20, and then incubated for 18h at 4°C with serum in 1:50, in 1% skim milk in PBS. Following further washes, the strips were incubated for 1h with goat anti-human immunoglobulin G (IgG)-biotin/avidin peroxidase (Sigma) conjugate (1:3,000). Following additional washes, the antigen-antibody complexes were obtained by incubation with an appropriate substrate: 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) containing 0.06% H₂O₂ (for the peroxidase conjugate).

Statistical analysis

The Kappa coefficient (k) was calculated to evaluate the agreement between the ELISA results for the serum and whole blood³⁰.

The Chi square test with significance set at $p < 0.05$ and meta-analysis were used in order to verify the correlation between the immunological test results and risk factors³¹.

The results of the seroepidemiological studies focusing on cysticercosis were sent to the health authorities of the municipality, in order to contribute to the collection of information on endemicity and on risk factors for transmission.

Ethical considerations

Ethical approval for the study was granted by the Research Ethics Committee of UFSC, Brazil, under protocol no. 211/03.

RESULTS

Data obtained from 877 questionnaires answered orally provided a demographic profile of this population regarding age, sex, residential location, education and information related to the epidemiology of teniasis/cysticercosis in the area, as shown on **Table 1**.

TABLE 1 - General characteristics of studied population.

General characteristics	Total of individuals (877)	
	n	%
Gender		
male	283	32.3
female	594	67.7
Type of dwelling		
rural	254	29.0
periurban	623	71.0
Education level		
illiterate	96	11.0
basic	631	71.9
others	150	17.1
Taenia sp. infection		
self	218	24.9
family member	232	26.5
non	427	48.6
Pig rearing		
confined (present)	134	15.3
loose (present)	53	6.0
past	107	12.2
non	583	66.5
Age	39.5 (5 to 71)	

The results for ELISA using whole blood were in agreement with those using serum samples ($k = 0.73$) up to one year after collection. A total of 850 samples of whole blood collected in filter paper were able to be analyzed by ELISA using Tcra-vf as antigens and positive reactions were obtained in 186 (21.9%) individuals. A total of 213 volunteers were asked to collect blood samples for use in the immunoblot assay (IB) confirmatory assay (186 positive for ELISA and 27 whose initial sample was considered inappropriate). The IB test was performed on 130 samples collected from people who showed up to provide a blood sample and 29 presented a positive reaction. Considering that these individuals are putative holders of cysticerci, analysis of their socioeconomic profiles was conducted in an attempt to relate infection with known factors that predispose human populations to cysticercosis.

The prevalence encountered in the population of Lages (SC) was 3.4%, since the immunoblot confirmatory test was positive in 29 samples out of 850 tested individuals. A significant correlation was determined among individuals tested positive by IB who practice both pig rearing and kitchen gardening ($p = 0.0364$).

DISCUSSION

The use of filter paper proved to be an appropriate option for field work where no facilities to collect vein blood or to obtain serum from whole blood are available. The data obtained were in agreement with results reported by Peralta et al²⁶ and Fleury et al²⁵, and thus epidemiological studies on cysticercosis can be successfully conducted using this method.

The results obtained using immunoglobulin G (IgG) eluted from dried blood were consistent with most of the IgG concentrations in the corresponding serum samples. The overall prevalence of 3.4%

encountered in this population is compatible with reports from other seroepidemiological surveys conducted in Brazil³².

Bragazza et al²⁰ reported a serum prevalence of 2.1% for cysticercosis in individuals from a rural population in the State of São Paulo, associated with the consumption of untreated water. Prestes-Carneiro et al³³ verified a frequency of 0.6% of anti-*Taenia solium* cysticerci, confirmed by the immunoblot method, in a rural settlement in the State of São Paulo. According to the authors, the index was considered low and was a consequence of continued investment in public health care and private institutions to improve the infrastructure services and education.

In this study, pig rearing and kitchen gardening were determined as risk factors associated with *Taenia solium* infection in the population studied ($p = 0.0364$). The highest prevalence (65% of the confirmatory IB) was verified in the areas known as Rancho de Tábua and Tributo, a rural and periurban area, respectively. Rancho de Tábua is an area where pigs are reared for domestic consumption and the transmission of *Taenia solium* to pigs requires access to human feces and human consumption of improperly cooked pork. These conditions are still very common in the developing world.

More than 80% of the population of Lages lives in urban areas, which account for only 10% of the area of the municipality³⁴. This intense urbanization results in crowded areas around the town center with poor sanitation and precarious housing and is commonly associated with low education and economic levels of the inhabitants. Tributo is a locality with the conditions necessary for the maintenance of teniasis/cysticercosis transmission. Its population lives on the border between the urban and rural areas, maintaining some rural habits, such as domestic rearing of a small number of pigs in the backyard fed with leftover food and often in free contact with domestic sewage.

This study was conducted by visiting houses or at community health centers during the day, when most men are at work and women, mostly housewives, are at home taking care of children. This explains the higher frequency of women in the population sample.

The findings provided data which will be useful to the authorities when planning interventions in routine healthcare practices and, more importantly, in preventive healthcare practices. Moreover, it was suggested that the positive results obtained in this study were registered in the medical records of each participant to improve medical care in cases of the appearance of related symptoms.

In conclusion, the local seroprevalence of cysticercosis verified among volunteers from rural and periurban areas of Lages, SC, was similar to that reported in others studies in Latin America. Among all the risk factors analyzed, pig rearing and kitchen garden were positively associated with cysticercosis markers.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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