

NEUROCYSTICERCOSIS

DETECTION OF IgG, IgA AND IgE ANTIBODIES IN CEREBROSPINAL FLUID, SERUM AND SALIVA SAMPLES BY ELISA WITH *TAENIA SOLIUM* AND *TAENIA CRASSICEPS* ANTIGENS

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ABSTRACT - We assayed samples of cerebrospinal fluid (CSF), serum and saliva from patients with neurocysticercoses, control group and individuals with other parasitoses, by ELISA with *Taenia crassiceps* vesicular fluid antigen (Tcra) and *Taenia solium* total antigen (Tso) for the detection of antibodies. The sensitivity for IgG-Tcra was 100% for CSF and serum, and 32.0% for saliva; and for IgG-Tso 100% for CSF, 80.0% for serum and 24.0% for saliva. Specificity was 100% for CSF and 80.0% for serum with both antigens, and 100% for saliva with Tcra and 87.5% with Tso. The sensitivity and specificity for IgA-Tcra was, respectively, 40.0% and 100% for CSF, 36.0% and 97.1% for serum, and 4.0% and 90.0% for saliva. IgE detection showed 24.0% sensitivity and 97.1% specificity for serum, with no detection in CSF samples. The search for antibodies revealed the presence of IgG > IgA > IgE in CSF, serum and saliva samples, with IgG being present in all phases of the disease, while IgA/IgE were more frequent in the inactive form.

KEY WORDS: cysticercosis, central nervous system, humoral immune response, IgG, IgE, IgA, *T. crassiceps*.

Neurocisticercose: detecção de anticorpos IgG, IgA e IgE em amostras de líquido cefalorraquidiano, soro e saliva por ELISA com antígenos de *Taenia solium* e *Taenia crassiceps*

RESUMO - Analisamos amostras de líquido cefalorraquidiano (LCR), soro e saliva de pacientes com neurocisticercose, grupo de controle e indivíduos com outras parasitoses, por ELISA com antígenos de líquido vesicular de *T. crassiceps* (Tcra) e salino total de *T. solium* (Tso) para a pesquisa de anticorpos. A sensibilidade para IgG-Tcra foi 100% para LCR e soro e 32,0% para saliva, e para IgG-Tso 100% para LCR, 80,0% para soro e 24,0% para saliva. A especificidade foi de 100% para CSF e 80,0% para soro com ambos os antígenos, e 100% para saliva com Tcra e 87,5% com Tso. A sensibilidade e especificidade para IgA-Tcra foi, respectivamente: 40,0% e 100% para LCR, 36,0% e 97,1% para soro, e 4,0% e 90,0% para saliva. A pesquisa de IgE mostrou 24,0% de sensibilidade e 97,1% de especificidade para soro, sem detecção nas amostras de LCR. A pesquisa de anticorpos revelou a presença de IgG > IgA > IgE no LCR, soro e saliva, com presença de IgG em todas as fases evolutivas da doença, enquanto que IgA/IgE foram mais frequentes na forma inativa.

PALAVRAS-CHAVE: cisticercose, sistema nervoso central, resposta imune humoral, IgG, IgE, IgA, *T. crassiceps*.

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Neurocysticercosis (NC), the presence of *Taenia solium* metacestodes in tissues, is the most frequent and severe parasitic infection of the central nervous system. Its distribution is universal, being frequent in developing countries in Latin America, Africa, Asia and India¹⁻⁵, and with cases reported in the United States due to the immigration of individuals coming from endemic areas⁶.

The diagnosis of NC is based on clinical, epidemiologic and laboratory approaches (neuroimaging and immunological methods). Since clinical diagnosis is hindered by the nonspecific and polymorphic symptomatology of NC, the detection of anti-*T. solium* antibodies in the cerebrospinal fluid (CSF) constitutes an important diagnostic element. One of the difficulties in such studies is obtaining parasites from the model of natural infection in swine. *Taenia crassiceps* metacestodes, found in rodents, have been described in natural and experimental infections, and have been used as an alternative source of antigen in immunological tests for NC^{2,7-11}.

Most of the antibodies found in CSF are intrathecally synthesized, with a smaller proportion coming from peripheral blood by blood-brain barrier rupture¹². Several authors have detected IgG antibodies in CSF and/or serum from patients with NC, especially when the parasite is in the phase of degeneration and there is an increased immune-inflammatory host response^{9,12-14}. Some authors have reported IgG, IgM, IgA, IgE and IgD antibodies in CSF and/or serum from patients with NC, without correlation between antibody class and clinical aspect of the disease^{13, 15, 16}. In parasitic infections, including the teniasis-cysticercosis complex, the level of total IgE can be high, many times without the identification of the specificity of the antibody^{17,18}.

The objective of the present study was to investigate the humoral immune response in NC by detection of IgG, IgA and IgE antibodies in CSF, serum and saliva samples from patients classified according the evolutionary phase of the disease, by ELISA using *T. solium* and *T. crassiceps* antigens.

METHOD

Samples

We studied 25 paired samples of CSF, serum and saliva from patients with NC (P), selected according to the General Protocol of Investigation of NC of the Center of Neurological Investigations, University Hospital, Faculty of Medicine of the University of São Paulo. The study was approved by the Ethics Committee for Analysis of Research Projects, Clinical Management of UHFMUSP, 072/97, in agreement with Resolution 196/96 of the National Council of Health, Ministry of the Health, Brazil.

The patients were classified as follows on the basis of neuroimaging data (computed tomography and/or nuclear magnetic resonance image): 15 (60%) with the active form (inflammatory process) and 10 (40%) with the inactive form (no inflammatory image, 4 of them with calcified cysts). More detailed: 8% of the patients with NC presented imaging exams without alterations (type I), 16% presented intact cysts (type II), 16% presented degenerating cysts with an inflammatory process (type III), 16% calcified cysts (type IV), and 44% more than two evolutionary forms (mixed type)¹⁹.

As a control group (C) we studied CSF samples from 10 individuals with other neurological disorders, and 35 serum samples and 7 saliva samples from presumably healthy individuals. The group of other parasitoses (OP) consisted of 23 serum samples with immunological tests reactive to other parasitoses, i.e., toxocaríasis (n=7), toxoplasmosis (n=6), Chagas' disease (n=5), and schistosomiasis (n=5).

Parasites and antigens

The parasites, the metacestode forms of *T. crassiceps* (ORF strain) and of *T. solium*, and the antigenic extracts of vesicular fluid of *T. crassiceps* (Tera) and total saline extract of *T. solium* (Tso) were obtained according to Bueno²⁰. Phenylmethylsulphonyl fluoride (Sigma Chem. Co., USA), 4×10^{-1} mM, was added to each antigenic extract.

Immunoenzymatic test (ELISA)

Polystyrene flat bottom plates were used (Costar Corporation, USA). For blockade we used 5% skim milk (Nestlé, Brazil) in 0.15 M NaCl with 0.05% Tween 20 for 2 hours at 37°C. We used Tera and Tso antigens (10

$\mu\text{g/ml}$ in 0.05 M carbonate-bicarbonate buffer, pH 9.6), CSF, serum and saliva samples (respectively 1:2, 1:100 and 1:2 in 1% milk), anti-human IgG-peroxidase conjugate (Biolab Diagnóstica SA, Brazil) and chromogen-substrate (1 g/l orthophenylenediamine and 1 ml/l H_2O_2 in 0.2 M citrate buffer, pH 5.0) in volumes of 100 μl /well. The material was incubated at 37°C for 1 h, except for the chromogen-substrate, which was incubated for 15 min. The reaction was blocked with 0.5 N H_2SO_4 and the absorbance was read at 492 nm.

For IgA and IgE antibody detection, the samples were pre-treated 1:2 (v/v) with RF-Absorbent (Behring Diagnostic Inc., Germany), previously diluted to 1:2 in distilled water.

The determination of the cut-off for IgG detection in CSF and serum samples was based on the analysis of the diagnostic efficiency of each test according to the Youden index²¹, calculated for values from the C group [mean + nsd (n -1.16 to +7.0)]. The other cut-off rates were defined as the mean of the C group plus 2 standard deviations.

RESULTS

The results obtained by ELISA with the Tcra and Tso antigens are presented in the Figure 1.

Table 1. Cut-off, sensitivity and specificity (%) with respective confidence intervals (CI), and Youden index of ELISA in the detection of IgG, IgA and IgE antibodies in CSF, serum and saliva samples using *Taenia solium* (Tso) and *Taenia crassiceps* (Tcra) antigens.

Ig Antigen	Sample	Cut-off	n*	Sensitivity (CI)	n**	Specificity (CI)	Youden Index
IgG Tso	CSF	0.078	25	100.0 (99.4-100.0)	10	100.0 (99.0-100.0)	1.0
	Sera	0.400	25	80.0 (72.0-88.0)	35	80.0 (73.2-86.8)	0.600
	Saliva	0.096	25	24.0 (15.5-32.5)	8	87.5 (75.8-99.2)	0.115
IgG Tcra	CSF	0.011	25	100.0 (99.4-100.0)	10	100.0 (99.0-100.0)	1.0
	Sera	0.097	25	100.0 (99.4-100.0)	35	80.0 (73.2-86.8)	0.800
	Saliva	0.172	25	32.0 (22.7-41.3)	9	100.0 (99.0-100.0)	0.320
IgA Tcra	CSF	0.010	25	40.0 (30.2-49.8)	4	100.0 (98.4-100.0)	0.400
	Sera	0.070	25	36.0 (26.4-45.6)	35	97.1 (94.3-99.9)	0.331
	Saliva	0.106	25	4.0 (0.1-7.9)	10	90 (80.5-99.5)	0
IgE Tcra	CSF	0.252	25	0	4	100.0 (98.4-100.0)	0
	Sera	0.135	25	24.0 (15.5-32.5)	35	97.1 (94.3-99.9)	0.211

*patient with NC; **control group

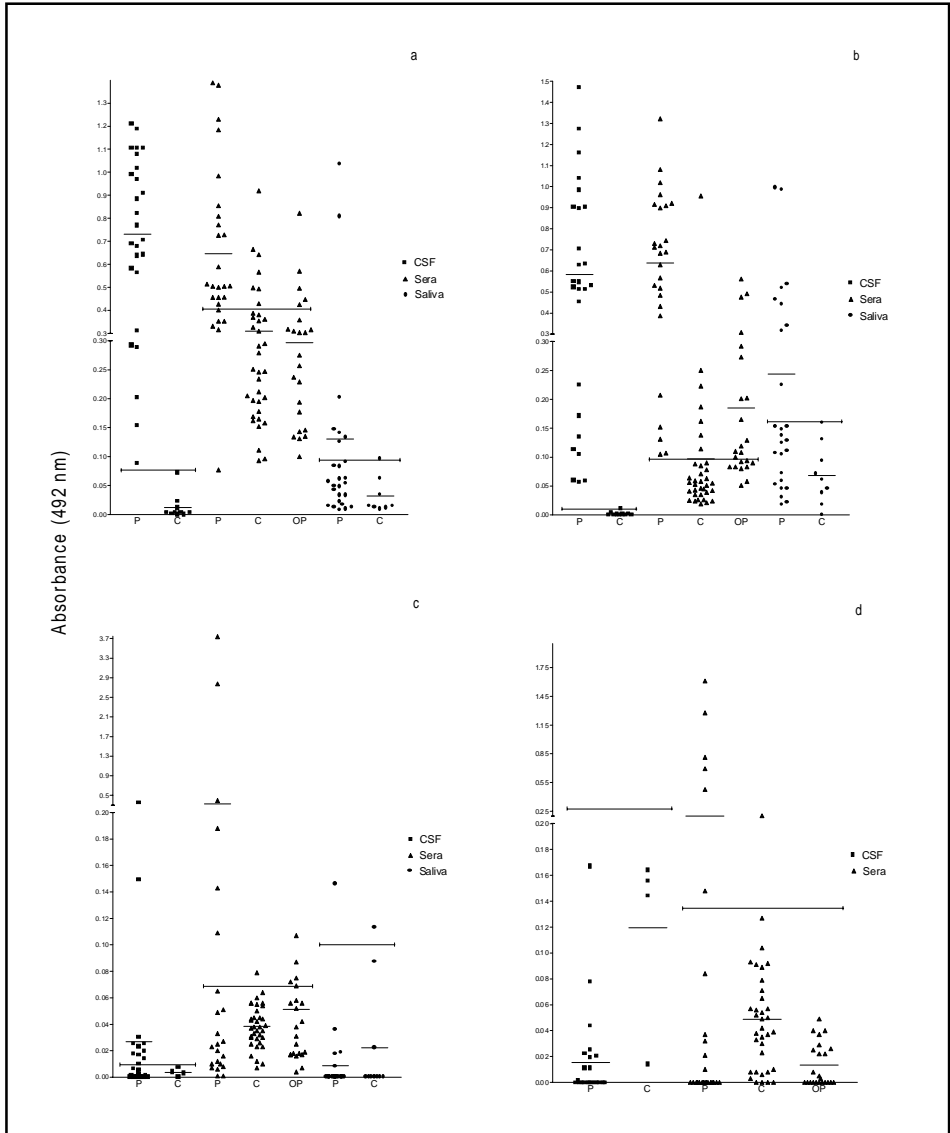


Fig 1. Results of CSF, sera and saliva samples from patients with neurocysticercosis (P), control group (C) and a group with other parasitosis (OP) obtained by ELISA using the total saline antigen of *Taenia solium* (a) and vesicular fluid antigen of *Taenia crassiceps* (b, c, d) for the detection of IgG (a,b), IgA (c) and IgE (d) antibodies [—]: cut-off.

Table 1 presents the indexes of the tests: cut-off, sensitivity and specificity, confidence intervals (95% probability), and Youden index.

In the OP group 5 (22%) samples were IgG-reactive with Tso antigen (toxoplasmosis, toxocaríasis and schistosomiasis) and more 9 (39%) other samples reacted with Tcra (plus Chagas' disease). IgA antibodies were detected in 5 (22%) serum samples (toxocaríasis, Chagas' disease and schistosomiasis).

Statistical analysis of the results by the Mann-Whitney test revealed a significant difference between the P group and the C/OP groups for IgG and IgE detection in CSF and serum samples ($p < 0.0389$). IgA detection did not show a significant difference between the P and C groups ($p > 0.5425$). The saliva samples showed a significant difference between the P and C groups only for IgG in the ELISA Tcra ($p = 0.0330$).

DISCUSSION

The biological parasite-host interactions involved in NC are complex because of the different evolutionary stages of the parasite and of the individual variations of the response of the host⁶. On the other hand, it is difficult to obtain appropriate antigens in sufficient amounts for the immunological tests from swine naturally infected with *Taenia solium* metacestode, of clandestine creation. The model of murine cysticercosis induced by *Taenia crassiceps*, of easy maintenance in the laboratory, appears as an alternative for obtaining parasites due to the antigens shared by *Taenia* species^{2,7-11}.

The classification of NC patients in the present study²² showed that only 60% had the active form of the disease, or cysts in degeneration and immune-inflammatory processes. Although 40% of the patients had the inactive form of the disease, the IgG detection showed sensitivity of 100.0% for CSF and serum and 32.0% for saliva with Tcra antigen, and 100.0% for CSF, 80.0% for serum and 24.0% for saliva with Tso antigen.

Similar results were related by VAZ, 1993²³, in ELISA with *T. solium* total antigen and *T. crassiceps* vesicular fluid antigen of LCR samples from patients with NC. Less sensitive results were obtained by other authors using *T. crassiceps* vesicular fluid antigen and CSF^{2,7} or *T. solium* total antigen and serum¹³. Kunz et al.²⁴, detected antibodies in the 14 serum samples analyzed by ELISA with antigen of vesicular fluid from *T. crassiceps* and *T. solium*, with higher reading intensity with *T. solium* antigen, similar to our results (Fig 1). Otherwise, other authors reported higher sensitivity than us for IgG detection in saliva samples^{25,26}.

The IgA detection with Tcra antigen revealed sensitivity and specificity, of 40.0% and 100.0% for CSF, 36.0% and 97.1% for serum, and 4.0% and 90.0% for saliva, respectively, while the IgE detection showed sensitivity of 24.0% and specificity of 97.1% for serum, and no CSF sample was reactive. Different results were reported by other authors, like Espinoza et al.¹³ using ELISA with *T. solium* total antigen for IgA detection, with positivity of 25% for serum and of 13% for CSF, and 3% positivity for IgE in serum and CSF. These discordant results reinforce the heterogeneity of the humoral immune response involved in the parasite-host interaction in NC. Acosta²⁶ did not find any difference in IgA detection in saliva samples between the group of patients with NC, the control group and the group with other neurological disorders by ELISA with *T. solium* total antigen. Although we used the Tcra antigen, we also did not find significant differences among the groups in CSF, serum or saliva samples ($p < 0.001$).

Five serum samples from patients with NC were negative for IgG detection with Tso antigen, but positive for Tcra. Two (40%) of them presented cysts in degeneration, 2 (40%) calcified cysts, and 1 (20%) a normal imaging exam. These results suggest a higher sensitivity of the Tcra antigen in the detection of serum antibodies in patients that do not present signs of inflammatory processes. Some authors have reported lower sensitivity of the immunological tests in patients with calcified cysts, independent of the antigen employed^{24, 27, 28}.

The IgA detection showed positivity in serum samples of 33% patients with the active form and in 40% patients with the inactive form, while IgE was detected in 20% and 30% of cases, respectively. The IgA and IgE detection in the serum samples showed higher positivity in patients with the inactive form, and these results can be justified by liberation of antigenic products during the death and degeneration of the parasite, with formation of antigen-antibodies immune complexes in patients in active form²⁹. Differently, other authors did not obtain a correlation between the antibody class and the clinical aspect of the disease^{13, 15, 16}.

The frequency of specific antibodies detected by ELISA, independently of the sample, presented immunoglobulins of the G, A and E classes, in this order of frequency, similar to those found by other authors¹³. However, other authors identified immunoglobulins of the G, M, E, A and D classes, in this order of frequency, in serum samples^{16, 30}. The presence of anti-*T. solium* IgG antibodies at a higher frequency in CSF samples than in serum and in saliva samples is indicative of a secondary immune response, and is justified by the chronic process of the disease¹³, and also by the presence of the antibodies, except for IgG, on the surface of the parasite and consequently absent as circulating antibodies¹⁵. However, some authors have suggested that the IgM, IgA and IgE antibodies are not important in the NC immune response^{12, 17}.

The specific antibodies of the IgG and IgA classes in patients with NC were more frequent in CSF, followed by serum and then by saliva. Only the specific antibody of the IgE class was more frequent in serum than in CSF. Acosta²⁶, had already reported that there was no significant difference between the results obtained with serum and saliva samples for IgG detection by ELISA with *T. solium* total antigen, with a better performance for CSF samples. The higher frequency of antibodies in CSF is expected due to the local immune response at the site of lesion and to the compartmentalization of the process.

The results obtained with the Tcra and Tso antigens reinforce that the parasites share important antigenic determinants and in enough concentrations for use in the immunodiagnosis of NC, using CSF and serum samples for IgG detection, although confirmation of positive results is needed for serum samples because of the lower specificity. The Tcra antigen presented better efficiency and more homogeneous results in the detection of serum-specific antibodies, especially in the presence of calcifications and of a reduced immune-inflammatory response. We believe that the Tcra antigen, although heterologous, presents these advantages by being rich in components of vesicular fluid that probably involve soluble antigens of secretion and excretion. These antigens are also present in *T. solium* cysticerci, but at lower concentrations than in the membrane components and scolex, due to the disruption of the vesicles in the process of obtaining parasite.

The search for antibodies in patients with NC revealed the presence of immunoglobulins of the G > A > E classes in CSF, serum and saliva samples, in this order of frequency. We also observed the presence of IgG in all different evolutionary phases of the disease, while antibodies of the IgA and IgE classes were more frequent in the patients with the inactive form, with no degenerating cysts or immune-inflammatory processes, demonstrating the heterogeneity of the humoral immune response to NC. The study of these immunoglobulins in a larger number of samples from patients with NC in different clinical phases may be helpful for a better understanding of the parasite-host relationship.

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