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Detection of IgG in cerebrospinal fluid for diagnosis of neurocysticercosis: evaluation of saline and SDS extracts from *Taenia solium* and *Taenia crassiceps* metacestodes by ELISA and immunoblot assay

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Summary We compared saline (S) and sodium dodecyl sulphate (SDS) extracts from *Taenia solium* (homologous species – HO) and *Taenia crassiceps* (heterologous species – HE) metacestodes in order to detect IgG by ELISA and immunoblot assay (IBA) in cerebrospinal fluid (CSF) for the diagnosis of human neurocysticercosis (NC). CSF samples were obtained from 93 patients. Of these, 40 had NC, five had a diagnosis of probable NC, nine had central nervous system schistosomiasis or strongyloidiasis and 39 had other neurological alterations. Samples were analysed by ELISA and the results were compared with IBA in all samples with confirmed and probable NC diagnosis, in all samples with other central nervous system parasitic infection, and in 10 of those with another neurological alterations. ELISA sensitivity was 100%, 85%, 95% and 87.5% for the S-HO, S-HE, SDS-HO and SDS-HE extracts, respectively, and ELISA specificity was 100% for S-HO, S-HE, SDS-HO extracts and 97.9% for SDS-HE antigen. Immunodominant peptides detected by IBA were, by decreasing percentage of recognition: 64–68 and 45 kDa for S-HO; 108–114, 92–95, 64–68, 83 and 88 kDa for S-HE; 64–68, 108–114, 77 and 86 kDa for SDS-HO; and 108–114, 88 and 92–95 kDa for SDS-HE. Overall the homologous antigenic extracts showed higher sensitivity than the heterologous extracts in the diagnosis of NC in CSF samples. The heterologous extracts contained most of the immunodominant peptides presented in the homologous extracts, which are recognized by IgG antibodies in CSF samples.

keywords *Taenia solium*, *Taenia crassiceps*, cysticercosis, immunodiagnosis, ELISA, immunoblot assay

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Introduction

Human cysticercosis occurs after ingestion of *Taenia solium* eggs. Neurocysticercosis (NC) is the most common manifestation. It presents with polymorphic and non-specific symptoms, hence clinical and epidemiological data and images must be evaluated and specific antibodies in human cerebrospinal fluid (CSF) or serum samples must be detected to make a definite diagnosis (Antoniuk 1999; Garcia & Del Brutto 2000).

The metacestodes obtained from *T. solium* metacestodes have greatly facilitated immunodiagnostic tests for sero-epidemiology in endemic areas of cysticercosis (Larralde *et al*. 1990; Biondi *et al*. 1996). The antigens from murine *Taenia crassiceps* metacestodes, ORF strain (Freeman 1962), effectively substitute those from porcine parasites in

the immunodiagnosis of human NC in CSF (Larralde *et al.* 1990; Ferreira *et al.* 1997; Vaz *et al.* 1997).

ELISA has been largely employed in the diagnosis of NC. The use of different antigenic fractions of *T. solium* metacestodes, such as saline extract, vesicle fluid, crude alkaline, scolex or membrane extracts, has resulted in differences in sensitivity and specificity of the tests (Costa 1986). IgG, IgM, IgA and IgE have been detected by ELISA in CSF and serum samples (Correa *et al.* 1985; Costa *et al.* 1985; Espinoza *et al.* 1986; Melo *et al.* 1997).

The immunoblot assay (IBA) allows identification of specific antigenic peptides which are recognized by serum samples through an electrophoresis of parasite crude extracts, thus eliminating false-positive results by ELISA (Larralde *et al.* 1989). By IBA, immunodominant antigens were demonstrated when migrating from 8 to 155 kDa in CSF samples, and most were <100 kDa (Gottstein *et al.* 1986; Vaz *et al.* 1997). The glycoproteins described by Tsang *et al.* (1989) have been used in the diagnosis of NC (Garcia *et al.* 1994; Andriantsimahavandy *et al.* 1997; Jafri *et al.* 1998).

Our aim was to compare saline (S) and sodium dodecyl sulphate (SDS) extracts from *T. solium* (homologous – HO) and *T. crassiceps* (heterologous – HE) metacestodes for the diagnosis of NC by ELISA and IBA in CSF samples.

Materials and methods

Patients and CSF samples

This study received ethical approval from the Federal University of Uberlândia Ethical Committee. CSF samples were collected from 93 patients admitted to the Clinical Hospital of Federal University of Uberlândia, State of Minas Gerais, Brazil. From these patients, 40 had a diagnosis of established NC based on the clinical syndrome, epidemiological data and evidence of the parasite by computed tomography or magnetic resonance. These patients were also positive by ELISA, as described by Costa *et al.* (1982). The remaining 53 samples were distributed as follows: five patients with a diagnosis of probable NC, without confirmatory exams (two with cephalea, one with epilepsy, one with hydrocephalus and one with hemiparesis); nine patients with central nervous system infection caused by other parasitic agents (eight with *Schistosoma mansoni* and one with *Strongyloides stercoralis*); and 39 patients with other neurological disorders, such as epilepsy (13), cephalea (8), viral meningitis (7), behaviour disturbances (6), hydrocephalus (4) and cerebral vascular accident (1). All CSF samples analysed in the present investigation were obtained from a Biological Sample Collection from the Laboratory of Parasitology of the Federal University of Uberlândia. All these CSF samples were submitted to ELISA and all the samples with confirmed and probable NC diagnosis and 10 control samples were analysed by IBA.

Parasites

Taenia solium metacestodes were obtained from the muscles of naturally infected swine, washed in saline solution (0.15 M NaCl) four times and stored at –20 °C. *Taenia crassiceps* metacestodes, ORF strain, were maintained by intraperitoneal inoculation in AJ Snell mice. After 90 days, the parasites were collected from the peritoneal cavity, washed four times in saline solution and stored at –20 °C.

Preparation of antigens

Saline extracts from 50 homologous species (S-HO) and 200 heterologous species (S-HE) metacestodes were prepared as described by Costa *et al.* (1982). For SDS extraction, 10 ml of 0.01 M phosphate buffered saline (PBS), pH 7.2 were added to 50 homologous (SDS-HO) and 200 heterologous (SDS-HE) metacestodes. The materials were centrifuged at 2000 g for 10 min at 4 °C. The pellet was resuspended in 10 ml of PBS and again submitted to centrifugation three times for 10 min. Then it was homogenized with 2.5 ml of 0.5 M Tris–HCl pH 6.8, 20% glycerol and 4% SDS. These preparations were disrupted in an ice-bath using a glass tissue homogenizer for 20 min. Subsequently, the sample buffer consisting of 2.5 ml of 0.1 M Tris–HCl pH 6.8, 20% glycerol, 4% SDS and 0.2% bromophenol blue was added. Protein and polysaccharide measurements of each antigenic preparation were carried out according to Lowry *et al.* (1951) and Martirani *et al.* (1959), respectively.

ELISA

Polystyrene microplates (Interlab, São Paulo, Brazil) were incubated with S-HO, S-HE, SDS-HO and SDS-HE preparations at 10 μg/ml diluted in 0.06 M carbonate–bicarbonate buffer, pH 9.6. After incubation overnight at 4 °C, the microplates were washed three times for 5 min with PBS containing 0.05% Tween 20 (PBS-T) and incubated with the CSF samples diluted at 1 : 5 in PBS-T for 45 min at 37 °C. The conjugate (rabbit antihuman IgG, heavy chain specific, labelled with peroxidase, prepared as described by Wilson & Nakane 1978) was diluted at ideal titre of 150 for HO species and 100 for HE species in PBS-T and incubated for 45 min at 37 °C. The enzymatic substrate consisted of H_2O_2 plus *o*-phenylenediamine

(OPD) solution in 0.1 M citrate Na_2HPO_4 buffer pH 5.5. The reaction was stopped after 15 min with 25 μl/well of 1 M H_2SO_4 and the absorbances were determined in an ELISA reader (Titertek Plus, Flow Laboratories, USA) at 492 nm. The cut-off was established using media of three non-reactive CSF samples + 2 standard deviations (SD), as described by Bassi *et al.* (1991).

Electrophoresis and electrophoretic transfer

S-HO and S-HE antigens were diluted (v/v) in sample buffer; SDS-HO and SDS-HE antigens had already been prepared in this buffer. After boiling for 3 min at 100 °C, all preparations were submitted to electrophoresis in SDS–PAGE at 12% under non-reducing conditions, as described by Laemmli (1970). After SDS–PAGE, the gels were either stained by silver (Friedman 1982) or transferred to nitrocellulose membranes (0.45 μm; Hybond-C, Amersham, Life Science), as described by Towbin *et al.* (1979), by using a transfer apparatus (Multiphor II, Pharmacia – LKB).

Immunoblot assay

Nitrocellulose strips containing fractions of the S-HO, S-HE, SDS-HO and SDS-HE antigens were blocked with 5% non-fat milk in PBS-T for 2 h at room temperature and incubated overnight at 4 °C with CSF samples diluted at 1 : 5 in 1% non-fat milk in PBS-T (PBSTM). After washing with PBSTM, strips were incubated for 2 h at room temperature with the conjugate (rabbit antihuman IgG, heavy chain specific, labelled with peroxidase) diluted at 1 : 100 and 1 : 20 in PBSTM for HO and HE antigens, respectively. The strips were washed in PBS and developed with hydrogen peroxide and 3,3′-diaminobenzidine tetrahydrochloride (DAB-Sigma) for 3 min. The strips were washed with distilled water and the positive reactions were determined by the appearance of clearly defined brown blot. The relative molecular mass (M_r) of each recognized band was determined by comparison with the molecular markers (Sigma), containing myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), fructose-6-phosphate kinase (84 kDa), albumin (66 kDa), glutamic dehydrogenase (55 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), α-lactalbumin (14 kDa), and aprotinin (6 kDa).

Statistical analysis

Analysis of the data was performed using the Statistics for Windows software, Stat soft, Inc. 1993. To compare the frequency of antigenic markers recognized by IBA and to determine the frequency of detection of IgG anti-*T. solium* metacestodes in CSF samples by ELISA, the percentages of the revealed bands were tested by two proportions at the significance level of 5%.

Results

The protein concentrations of the antigenic extracts showed similar results for S and SDS extractions, for both *T. crassiceps* and *T. solium* metacestodes. The polysaccharide contents were more elevated in both HE extracts.

Figure 1 gives the absorbance values obtained by ELISA using the S and SDS extracts from *T. solium* and *T. crassiceps* metacestodes. As shown in Table 1, ELISA sensitivity was 100%, 85%, 95% and 87.5% for S-HO, S-HE, SDS-HO and SDS-HE extracts, respectively, and ELISA specificity was 100% for S-HO, S-HE, SDS-HO extracts and 97.9% for SDS-HE antigen.

Figures 2 and 3 illustrate the results of the immunoblots of CSF samples from 10 patients with NC using S (HO and HE) and SDS (HO and HE) extracts, respectively.

Figure 4 shows the frequency of the antigenic bands recognized by 40 CSF samples from patients with NC, by IBA using the different antigenic extracts. Immunodominant peptides detected by IBA in decreasing percentage of recognition were: 64–68 (60%) and 45 kDa (45%) for S-HO; 108–114 (70%), 92–95 (55%), 64–68 (50%), 83 (50%) and 88 kDa (42.5%) for S-HE; 64–68 (65%), 108–114 (55%), 77 (47.5%) and 86 kDa (40%) for SDS-HO; and 108–114 (92.5%), 88 (82.5%) and 92–95 kDa (42.5%) for SDS-HE extracts.

The five samples of CSF from patients with presumptive diagnosis of NC recognized markers of 26–28 (20%), 45 (20%) and 64–68 kDa (40%) in the extract S-HO; 88 (20%) and 108–114 kDa (40%) in S-HE and 64–68 (40%) and 77 kDa (20%) in SDS-HO extracts. In the group of patients with other central nervous system infections or other neurological alterations, none of the samples recognized any antigenic markers when the homologous or heterologous extracts were tested.

Table 2 compares the results obtained in both tests with regard to the various antigenic extracts. High indices of positive concordance (80.0–87.5%) were observed between the tests. HO extracts did not present any simultaneously non-reactive sample for both tests, while 2.5% of the samples were simultaneously negative for both heterologous extracts (S-HE and SDS-HE). Discordant results were seen in a wide range variation from 0% to 17.5%, depending on the typed extract analysed. For the homologous extracts, IBA+ and ELISA– results were found in 0% and 7.5% for S and SDS extracts, respectively. On

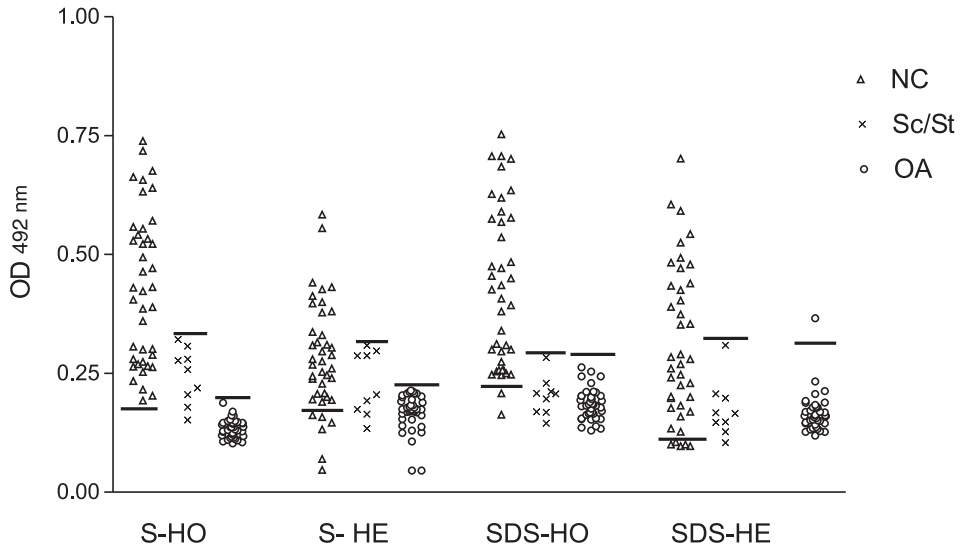

Figure 1 Results of CSF samples from 40 patients with NC, eight with schistosomiasis and one with strongyloidiasis (Sc/St) group and a group of 39 patients with other neurological alterations (OA) obtained by ELISA using the S and SDS extracts from *T. solium* (HO) and *T. crassiceps* (HE) metacestodes. (–) Cut-off, OD: optical density.

Table 1 ELISA sensitivity and specificity for the detection of IgG antibodies in CSF samples from 40 patients with NC and 48 samples from patients with other neurological alterations (n = 39) and central nervous system schistosomiasis or strongyloidiasis (9), using *T. solium* (homologous) and *T. crassiceps* (heterologous) metacestode extracts

Extracts	Sensitivity (n = 40)	Specificity (n = 48)
S-HO	100	100
S-HE	85.0	100
SDS-HO	95.0	100
SDS-HE	87.5	97.9

S: Saline; SDS: sodium dodecyl sulphate.

the other hand, the pattern of discordance for the heterologous extracts was of 12.5% and 10.0%, respectively, for S and SDS preparations. IBA– and ELISA+ results for homologous extracts achieved higher rates of discordance (S = 15.0%; SDS = 17.5%) than for heterologous extracts (S = 5%; SDS = 0%).

Discussion

The antigenic extraction of *T. solium* and *T. crassiceps* by SDS was studied for the first time in the diagnosis of human NC. The extracts were easily obtained and the different batches of these antigenic preparations proved to be reproducible. Protein concentrations of the SDS-HO and SDS-HE extracts were constantly higher than those of S-HO and S-HE extracts.

ELISA has been widely used in the diagnosis of human NC with high sensitivity and good specificity. In areas not endemic for schistosomiasis and human hydatid disease, this test is highly specific (Diwan *et al.* 1982). The use of CSF is more reliable, because the reactivity in serum samples for the diagnosis of cysticercosis does not check the location in CNS (Andriantsimahavandy *et al.* 1997). In our study none of the CSF samples from patients with other central nervous system parasitic infections was reactive, and none of the samples from patients with other neurological alterations recognized any antigenic marker in the antigenic extracts tested by ELISA. The sensitivity of the test was also very high, and only one sample that did not present reactivity by this test came from a patient with NC with intraparenchymal calcifications of CNS.

When using IBA, 15 antigenic markers (8, 12–13, 18, 24, 26–28, 39–42, 45, 54, 64–68, 77, 83, 86, 88, 92–95 and 108–114 kDa) were recognized in the studied extracts by CSF samples from patients with NC. Evaluating the heterogeneity of the immune response from patients with NC, the results were similar to those found by Vaz *et al.* (1997), where eight (98–92, 56–52, 72–68, 120, 155, 98–94, 76 and 115–108 kDa) immunodominant peptides in the HO extracts and five (72, 62, 42, 72–68 and 95–92 kDa) peptides in the HE extracts were recognized by CSF samples. The antigenic markers we identified (8 and 26 kDa) have a frequency of recognition <30%, and were

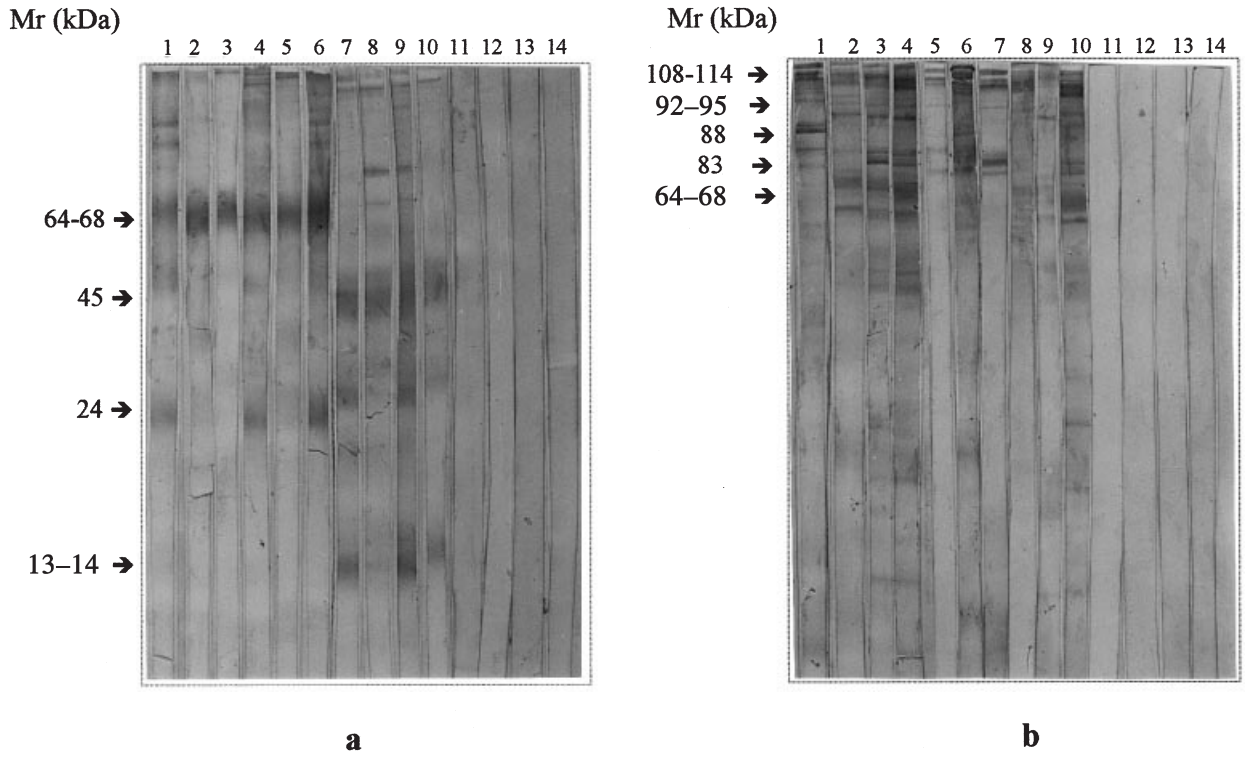

Figure 2 Western blotting of 10 CSF samples of patients with NC against extract S of *T. solium* metacestodes – S-HO (a) and extract S of *T. crassiceps* metacestodes – S-HE (b). The peroxidase-conjugated rabbit IgG antihuman IgG (heavy chain specific, 1 : 100 for S-HO and 1 : 20 for S-HE). The positions of relative molecular mass (M_r) of each recognized band was determined by comparison with the molecular markers (Sigma, USA).

described by Gottstein *et al.* (1986) and Ev *et al.* (1999) with sensitivity and specificity of 100% for the enzyme-linked immunoelectrotransfer blot (EITB). The 12–13, 18, 24 and 39–42 kDa bands identified in our study correspond to four of seven glycoproteins described by Tsang *et al.* (1989). Two of them (13 and 18 kDa) were also recently described by Ev *et al.* (1999). We found that the frequencies of recognition of these four markers were relatively low, with indexes of 32.5%, 30%, 25% and 7.5%, respectively, detected in the HO extracts. However, it should be pointed out that we used any system of glycoprotein purification.

The 64–68 and 45 kDa bands were reported to be specific for the diagnosis of human NC in CSF samples by Katti & Chandramukhi (1991). In our work, the 64–68 kDa marker was one of the immunodominant antigens in the S-HO, S-HE and SDS-HO extracts. When analysing CSF and serum of patients with NC, Simac *et al.* (1995) observed that bands of 60–75 kDa were recognized in CSF only. Vaz *et al.* (1997), using vesicular fluid of *T. solium* metacestodes, observed that bands of 72–68 kDa were recognized in 69.6% of CSF samples with NC analysed. The 45 kDa marker was only one of the immunodominant antigens in S-HO extract. The 92–95 kDa marker corresponds to a protein of the B antigen (Guerra *et al.* 1982) and was only immunodominant in the HE extracts.

The 108 kDa band was described as specific for the diagnosis of human NC in serum samples by using vesicle fluid of *T. solium* metacestodes, and the 88 kDa band of *T. crassiceps* metacestodes was considered discriminative for cysticercosis in a comparative analysis among the antigenic markers of HO and HE extracts, for cysticercosis and human hydatid disease (Larralde *et al.* 1989). Our results demonstrated that the 88 kDa band was one of the immunodominant antigens and exclusively recognized in the HE extracts. The 108–114 kDa band was one of the immunodominant antigens in the S-HE, SDS-HO and SDS-HE extracts and therefore the recognition of this marker was elevated for the HE species. Using vesicular fluid of *T. solium* metacestodes, Vaz *et al.* (1997) obtained 52.2% of frequency for 108–115 kDa marker in CSF samples.

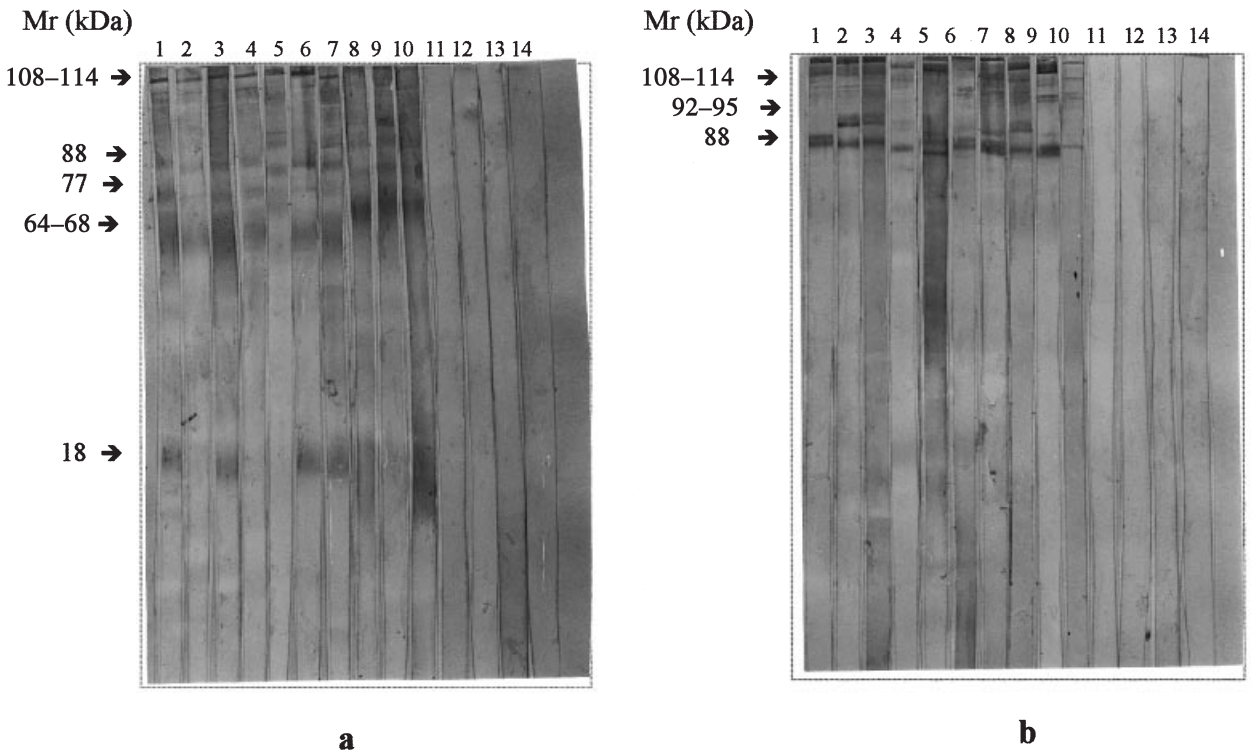

Figure 3 Western blotting of 10 CSF samples of patients with NC against extract SDS of *T. solium* metacestodes – SDS-HO (a) and extract SDS of *T. crassiceps* metacestodes – SDS-HE (b). The peroxidase-conjugated rabbit IgG antihuman IgG (heavy chain specific, 1 : 100 for SDS-HO and 1 : 20 for SDS-HE). The positions of relative molecular mass (M_r) of each recognized band was determined by comparison with the molecular markers (Sigma, USA).

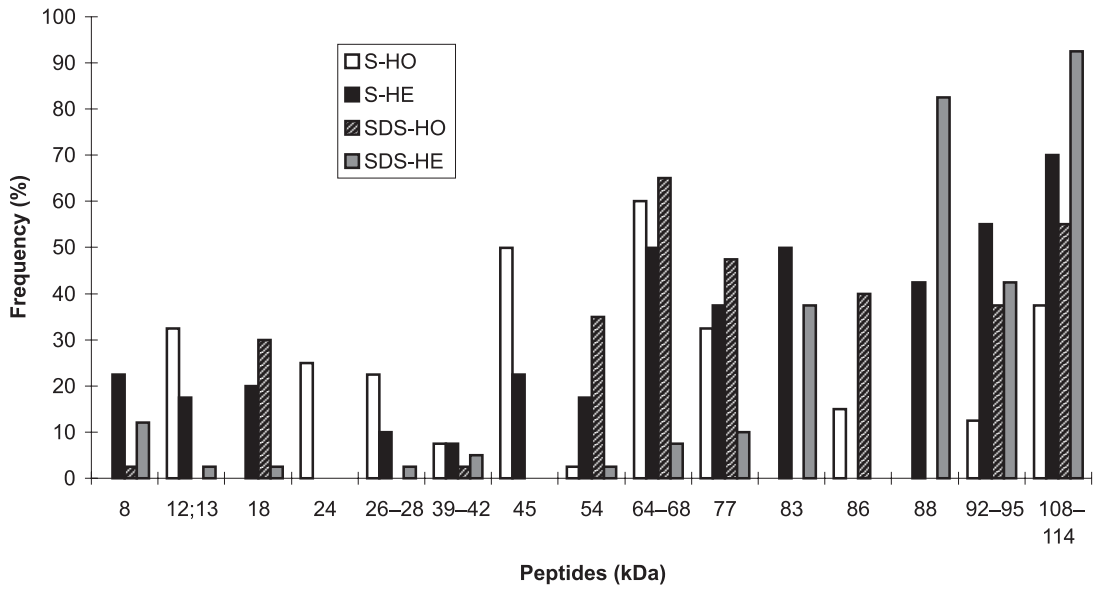

Figure 4 Frequency of antigenic markers (kDa) recognized by 40 CSF samples from patients with NC, diluted at 1 : 5, by IBA for detection of IgG anti *T. solium* metacestodes; using the S and SDS extracts from *T. solium* (HO) and *T. crassiceps* (HE) metacestodes.

Table 2 Comparison results of the IBA and ELISA in 40 CSF samples from patients with NC, using *T. solium* (homologous) and *T. crassiceps* (heterologous) metacestode extracts

Extracts	IBA+ and ELISA+	IBA+ and ELISA–	IBA– and ELISA+	IBA– and ELISA–
S-HO	34 (85.0)	0 (0.0)*,†	6 (15.0)‡	0 (0.0)
S-HE	32 (80.0)	5 (12.5)*	2 (5.0)	1 (2.5)
SDS-HO	30 (75.0)	3 (7.5)	7 (17.5)§	0 (0.0)
SDS-HE	35 (87.5)	4 (10.0)†	0 (0.0)‡, §	1 (2.5)

* S-HO × S-HE, $P < 0.05$.
† S-HO × SDS-HE, $P < 0.05$.
‡ S-HO × SDS-HE, $P < 0.05$.
§ SDS-HO × SDS-HE, $P < 0.05$.
Values in brackets denote percentages of concordance or discordance between the two immunoassays.

We compared the methods for antigenic extraction, S and SDS, with regard to frequency of immunodominant antigenic bands recognized by antibodies in the CSF samples studied. The 45 kDa band for the HO preparations was only recognized in the S extract, demonstrating that the SDS denaturating action made unfeasible the recognition of the respective marker in the SDS-HO extract. In this preparation, on the other hand, there was no interference in the recognition of 64–68 kDa band, because it was immunodominant in both extracts. The 108–114, 77 and 86 kDa bands presented a high recognition index in the SDS extract and therefore the denaturation process might have improved the exhibition of these epitopes.

With regard to the HE preparations, the antigenic markers of 83 and 88 kDa were exclusively recognized in the HE extracts, and the 83 kDa band was one of the immunodominant antigens in the S extract. The 88 kDa band was detected in both extracts, with 42.5% of recognition in the S-HE extract and 82.5% in the SDS-HE extract. Therefore, SDS increased the recognition of this band as well as the recognition of the 108–114 band. The 64–68 kDa band presented a high recognition frequency in the S-HE extract (50%), while the index in the SDS-HE extract was only 7.5%; therefore, the denaturation of this component by SDS, in this extract, decreased its capacity of being recognized by IgG antibodies.

The group of CSF samples from patients with presumptive diagnosis of NC recognized antigenic markers in three out of four studied extracts. However, the diagnosis in at least three of these patients has not been confirmed yet.

Overall the homologous antigenic extracts were more sensitive than the heterologous extracts in the diagnosis of NC in CSF samples. Also, the heterologous extracts contained the majority of the immunodominant peptides presented in the homologous extracts, which are recognized by IgG antibodies in CSF samples. The SDS preparations increased the recognition of some antigenic markers, such as the 108–114, 77 and 86 kDa bands in the SDS-HO extract, the 108–114 kDa band and mainly the 88 kDa marker in the SDS-HE extract. Therefore, SDS provided better yield in the antigenic preparations.

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