Serodiagnosis of human neurocysticercosis using antigenic components of *Taenia solium* metacestodes derived from the unbound fraction from jacalin affinity chromatography

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The aim of the present study was to analyse Taenia solium *metacestode antigens that were derived from the unbound fraction of jacalin affinity chromatography and subsequent tert-octylphenoxy poly(oxyethylene)ethanol Triton X-114 (TX-114) partitioning in the diagnosis of human neurocysticercosis (NCC). Immunoassays were designed to detect T.* solium-*specific IgG antibodies by ELISA and immunoblot. Serum samples were collected from 132 individuals who were categorised as follows: 40 had NCC, 62 presented* Taenia *spp or other parasitic diseases and 30 were healthy individuals. The jacalin-unbound ($J_{unbound}$) fraction presented higher sensitivity and specificity rates than the jacalin-bound fraction and only this fraction was subjected to subsequent TX-114 partitioning, resulting in detergent ($DJ_{unbound}$) and aqueous ($AJ_{unbound}$) fractions. The ELISA sensitivity and specificity were 85% and 84.8% for $J_{unbound}$, 92.5% and 93.5% for $DJ_{unbound}$ and 82.5% and 82.6% for $AJ_{unbound}$. By immunoblot, the $DJ_{unbound}$ fraction showed 100% sensitivity and specificity and only serum samples from patients with NCC recognised the 50-70 kDa* T. solium-*specific components. We conclude that the $DJ_{unbound}$ fraction can serve as a useful tool for the differential immunodiagnosis of NCC by immunoblot.*

Key words: neurocysticercosis - *Taenia solium* - jacalin - Triton X-114 - diagnosis

Neurocysticercosis (NCC) is caused by infection with *Taenia solium* metacestodes and is an important cause of neurological diseases worldwide. NCC is commonly associated with seizures, headaches and focal neurological deficits and can have such sequelae as epilepsy, hydrocephalus and dementia (Agapejev 2011, Carabin et al. 2011). This disease is distributed globally, particularly in Central and South America, India, Sub-Saharan Africa, East Asia, Eastern Europe and other developing countries (Garcia et al. 2010, Afonso et al. 2011, Prasad et al. 2011, Yanagida et al. 2012).

The diagnosis of NCC on clinical grounds is difficult because its signs and symptoms are non-specific. An accurate diagnosis requires a combination of clinical, epidemiologic, imaging and immunologic information (Nash & Garcia 2011). Imaging information, although very sensitive and specific, is not always available. For these reasons, immunoassays that are standardised for a proper diagnosis of NCC will lead to better clinical management of NCC patients because the immunoassays support diagnoses for patients whose clinical and imaging profiles are compatible with NCC (Michelet et al. 2011). Among various immunological methods, the ELISA and immunoblot are the most frequently used to detect antibodies against *T. solium* antigens in serum or cerebrospinal fluid samples, but these tests have shown different degrees of sensitivity and specificity, depending on the method of antigen preparation used (Shiguekawa et al. 2000, Barcelos et al. 2007, Deckers & Dorny 2010, Gonçalves et al. 2010, Lee et al. 2011, Michelet et al. 2011, Ferrer et al. 2012).

Jacalin, a major protein from the seeds of the jackfruit *Artocarpus integrifolia*, is a tetrameric two-chain lectin with a molecular mass of 60-66 kDa. This glycoprotein binds only to O-glycosidically linked oligosaccharides and prefers to bind galactosyl (β-1,3) N-acetylgalactosamine.

Lectins are proteins that possess at least one non-catalytic domain, which binds reversibly to a specific monosaccharide through hydrogen bonds, metal coordination, van der Waals interactions and hydrophobic interactions (Kabir 1998, Wu et al. 2003). This sugar-binding property of lectins also confers the capacity to be isolated by affinity chromatography on matrices containing their specific sugar ligand. Affinity separation techniques are based on biospecific molecular interactions. Therefore, they are extremely powerful tools for the isolation of valuable biological macromolecules (Jeyaprakash et al. 2005).

A solution of the nonionic surfactant Triton X-114 (TX-114) is homogeneous at 0ºC but separates into detergent (D) and aqueous (A) phases above 20ºC. The efficiency of this separation increases with temperature

and is sensitive to the presence of other surfactants. Integral membrane proteins of an amphiphilic nature are recovered in the D phase and hydrophilic proteins are found exclusively in the A phase (Bordier 1981). *T. solium* metacestode proteins recovered in the D phase have shown good results by ELISA and immunoblot for the diagnosis of NCC, but antigens purified using this technique alone have also shown some cross-reactivity, especially with *Echinococcus granulosus* by ELISA (Machado et al. 2007).

The major aim of this study was to isolate the antigenic components from a saline extract of *T. solium* metacestodes by jacalin affinity chromatography followed by TX-114 partitioning and to evaluate the antigenicity degree of these fractions in the detection of IgG antibodies by ELISA and immunoblot for the diagnosis of human NCC. After sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, our findings demonstrated that the jacalin-unbound ($J_{unbound}$) fraction showed to be more specific than the jacalin-bound ones and therefore, only this fraction was subjected to TX-114 partitioning.

SUBJECTS, MATERIALS AND METHODS

Serum samples - Serum samples were collected from 132 subjects who were selected by the Laboratory of Clinical Analysis of the Clinical Hospital (Groups 1 and 2) and the Laboratory of Parasitology (Group 3) of the Federal University of Uberlândia in the state of Minas Gerais (MG), Brazil. Group 1 was composed of 40 patients who had been diagnosed with definitive NCC based on the presence of clinical symptoms, epidemiological data, positive immunological tests and evidence of the parasite by computerised tomography, as follows: (i) clinical syndrome: all patients presented at least one type of clinical manifestation that was suggestive of NCC, including epilepsy (55%), cephalea (50%), dizziness (27.5%), dementia (12.5%), faintness (10%) and hydrocephalus (2.5%) and no signs or symptoms that were suggestive of the presence of metacestodes in other organs were present; (ii) epidemiological data: all patients came from or lived in an area where cysticercosis is endemic, as previously described (Barcelos et al. 2012), in addition to at least two instances of household contact with *T. solium* infection; (iii) immunological diagnosis: cerebrospinal fluid samples were positive for anti-cysticercal IgG antibodies by ELISA; (iv) cerebral computerised tomographic findings: all patients presented evidence of the parasite by neuroimaging with the following classifications based on Sotelo et al. 1985: eight (20%) vesicular, 15 (37.5%) vesicular/calcified and 17 (42.5%) calcified metacestodes. According to the Del Brutto diagnostic criteria, all patients from Group 1 had a definitive diagnosis; 29 (72.5%) had the absolute criteria and 11 (27.5%) presented two major plus one minor or epidemiologic criteria (Del Brutto 2012). Of the patients who presented with the active form of NCC (n = 23), 16 (70%) had the absolute criteria, whereas of those who presented with calcified lesions (n = 17), 13 (76%) had the absolute criteria.

Group 2 was composed of 62 patients who were inflicted with *Taenia* spp adult intestinal worms (10) and other parasites (52), according to the following distribution: *Ascaris lumbricoides* (6), *E. granulosus* (10), *Enterobius vermicularis* (6), *Giardia lamblia* (4), hookworm (6), *Hymenolepis nana* (4), *Schistosoma mansoni* (10), *Strongyloides stercoralis* (4) and *Trichuris trichiura* (2). With the exception of the *E. granulosus*-infected patients, all members of Group 2 were diagnosed by the ether sedimentation technique for routine stool examinations, as described by Ritchie (1948). The *E. granulosus*-infected patients were diagnosed by clinical assessment as well as by imaging and histopathological techniques. Group 3 was composed of 30 healthy volunteers, based on their clinical records. Although they came from areas that are endemic for cysticercosis, none of them presented any evidence of household contact with *T. solium* infection or a previous history of taeniasis or cysticercosis. In addition, three faecal samples from these individuals tested negative by the parasitological methods of Lutz (1919) and Baermann (1917).

Ethics - This study was approved by the Research Ethical Committee of the Federal University of Uberlândia, MG.

Parasites - Fresh *T. solium* metacestodes were collected from infected pig muscle and carefully dissected from the host tissues, washed repeatedly and stored at -20ºC. The saline extract was prepared as described by Costa et al. (1982), with modifications. Briefly, 50 metacestodes were disrupted in 5 mL distilled water in an ice bath for 5 min, homogenised for 4 min and subsequently submitted to ultrasonic treatment (Thornton, Inpec Electronics, Valinhos, São Paulo, Brazil) at 40 kHz for four cycles of 30 s each in an ice bath. After isotonisation in 5 mL of 0.3 M NaCl, the mixture was again submitted to ultrasonic treatment as described above and then stirred at 4ºC for 2 h and centrifuged at 12,400 *g* and 4ºC for 30 min (Du Pont SORVALL Products, Newtown, Connecticut, USA). The supernatants were analysed for protein content as described by Lowry et al. (1951) and stored in aliquots at -70ºC until use.

Isolation of jacalin-reactive components from saline extracts of T. solium metacestodes - The jacalin-reactive components were isolated according to Hermanson et al. (1995) with some modifications. First, the glycosylated proteins were isolated from saline extracts of *T. solium* metacestodes (12 mg total protein) by affinity chromatography using 5 mL jacalin immobilised on cross-linked 4% agarose (Sigma-Aldrich Chemical Co, St. Louis, Missouri, USA) previously equilibrated with phosphate-buffered saline (PBS). The column was locked and incubated overnight under continuous slow agitation at 4ºC. The unbound material was washed with PBS and the jacalin-bound components were eluted with 0.4 M D-galactose (Sigma) at a flow rate of 0.5 mL/min, monitored by absorbance at 280 nm and collected in 2 mL fractions. The jacalin-bound and $J_{unbound}$ fractions were independently concentrated using a stirred ultrafiltration cell (Amicon, YM-10, WR Grace & Co) and the jacalin-bound fraction was dialysed against PBS. The protein content of each antigen preparation was determined according to Lowry et al. (1951) and corresponded to 0.5 mg/mL for jacalin-bound and 3 mg/mL for $J_{unbound}$.

TX-114 partitioning - The D and A phases were obtained from chromatography batches containing 6 mg of total protein from the J$_{unbound}$ fraction using TX-114 (Sigma), as described by Machado et al. (2007). J$_{unbound}$ was diluted in 1.7 mL of Tris-buffered saline (10 mM Tris-HCl, pH 7.4, 150 mM NaCl) and 1% TX-114. This mixture was incubated at 0ºC for 10 min and layered carefully on a sucrose mixture (5.5 mL Tris-buffered saline plus 6% sucrose and 0.06% TX-114). The mixture was incubated at 37ºC for 10 min, followed by centrifugation at 3,000 *g* and 25ºC for 10 min, after which the upper phase was collected, 1% TX-114 was added and the mixture was incubated at 0ºC for 10 min. This solution was again layered carefully on the sucrose mixture used previously, incubated at 37ºC for 10 min and centrifuged at 3,000 *g* and 25ºC for 10 min. The supernatant was used to obtain the A phase and the pellet was used to obtain the D phase. The recovered supernatant was rinsed with 2% TX-114 in a separate tube without a sucrose cushion, incubated at 0ºC for 10 min and then 37ºC for 10 min and centrifuged at 3,000 *g* and 25°C for 10 min. The supernatant of this solution consisted of the A phase. The fractionated components (the DJ$_{unbound}$ and AJ$_{unbound}$ fractions) were precipitated (1:2; v/v) in cold acetone at 4ºC overnight and centrifuged at 3,000 *g* and 4ºC for 30 min. The supernatants were discarded and the precipitates were resuspended in 1 mL Tris-buffered saline. The protein content of both DJ$_{unbound}$ and AJ$_{unbound}$ antigen preparations was determined according to Lowry et al. (1951).

SDS-PAGE - The *T. solium* metacestode fractions were diluted (v/v) in sample buffer and after boiling at 98ºC for 3 min, all antigen preparations and molecular weight markers (Sigma) were subjected to 12% SDS-PAGE under non-reducing conditions, as described by Laemmli (1970), using an electrophoresis apparatus (Omniphor, England). The fractions and molecular weight markers were visualised by silver staining according to Friedman (1982) and the relative molecular masses were estimated using a logarithmic plot of the migration of a set of molecular weight standards, which was included in each gel.

ELISA - ELISAs for all extracts were carried out according to Machado et al. (2007). Briefly, polystyrene microplates (Interlab, Brazil) were coated with each extract at concentrations of 10 μg/mL in 0.06 M carbonate bicarbonate buffer, pH 9.6. These preparations were incubated overnight at 4ºC in a final volume of 50 μL/well. The microplates were washed three times (5 min each wash) with PBS containing 0.05% PBS-Tween 20 (PBS-T). The serum samples were diluted 1:200 in PBS-T, added to the microplates and incubated for 45 min at 37ºC. After washing as described above, peroxidase-conjugated goat anti-human IgG (Fc specific, Sigma) was added at a dilution of 1:1000 in PBS-T for all antigens and incubated for 45 min at 37ºC. The assay was read after washing by adding the enzymatic substrate solution, which consisted of H$_2$O$_2$ and ortho-phenylenediamine in 0.1 M citrate phosphate buffer, pH 5.5, for 15 min followed by 25 μL/well of H$_2$SO$_4$ (2 N) to stop the reaction. Optical densities (OD) were determined at 492 nm in an ELISA plate reader (Tp Reader, Thermoplate, China). The cut-off values were established using the mean OD of three non-reactive serum samples plus two standard deviations. The reactivity index (RI) was calculated according to Pardini et al. (2002). The data were subjected to "two-graph receiver operating characteristic" analysis, which is a plot of the test sensitivity and specificity against the threshold (cut-off) value, assuming the latter is an independent variable. A cut-off value was calculated for each antigen as the intersection point of the two graphs (Greiner et al. 1995). All serum samples with RI values > 1.1 were considered positive.

Immunoblot - Immunoblot assays were performed on all ELISA-reactive serum samples according to Machado et al. (2007). Antigen preparations were subjected to electrophoresis and transferred to nitrocellulose membranes (0.45 μm, Sigma), as previously described by Towbin et al. (1979), using a transfer apparatus (Omniphor, England). The nitrocellulose strips were blocked with 5% non-fat milk in PBS-T (PBS-TM) for 2 h at room temperature (RT) and incubated with serum samples diluted 1:50 in 1% PBS-TM overnight at 4ºC. After six washes with 1% PBS-TM, the strips were incubated for 2 h at RT with peroxidase-labelled goat IgG anti-human IgG (whole molecule, Sigma) diluted 1:1500 in 1% PBS-TM. The strips were washed in PBS and developed for 3 min in the substrate solution, which contained hydrogen peroxide and 3,3-diaminobenzidine tetrahydrochloride (Sigma) in PBS. The reaction was stopped by washing with distilled water and positive reactions were identified by the appearance of clearly defined bands. The relative molecular masses of the recognised bands were determined by comparison with molecular markers (Sigma) and the serum samples were considered positive when immunodominant components with a molecular weight of 50-70 kDa were recognised, according to Machado et al. (2007).

Statistical analysis - The geometric means of titres (GMT) of the RI for the ELISA data were calculated and compared for each group of serum samples and antigenic extracts using Student's *t* test. To determine if the proportion of positive samples within each group was significantly different from those of the other groups, the chi-square test was applied. Differences were considered significant when p < 0.05. Sensitivity, specificity and diagnostic efficiency (DE) were calculated according to Barbieri et al. (1998) and the Youden Index (YI) was determined as described by Youden (1950).

RESULTS

Analysis of the extracts in SDS-PAGE - Fig. 1 shows the affinity chromatography and electrophoretic profile of the saline extract and fractions. The J$_{unbound}$ fraction showed various bands by SDS-PAGE (87, 78, 70-50, 45, 37 and 24 kDa) and the jacalin-bound fraction showed five bands (94, 80, 32, 28 and 14 kDa). The protein yield from this process was 75.4% for the unbound fraction and 25.5% for the bound fraction. In the ELISA, the J$_{unbound}$ fraction was found to be more specific than the jacalin-bound fraction in terms of detection of IgG towards *T. solium*. Thus, only J$_{unbound}$ was subjected to TX-

114 partitioning. The protein yield from this process was 28.5% for the DJ phase and 55.5% for the AJ phase.

Fig. 2 shows the electrophoretic profile of the different antigenic fractions extracted from *T. solium* metacestodes with TX-114. The samples were separated by SDS-PAGE and stained with silver. Bands of 70-50, 48, 40 and 14 kDa were visualised in the DJ$_{unbound}$ fraction and bands of 85, 66, 50, 45 and 24 kDa were detected in the AJ$_{unbound}$ fraction.

Sensitivity and specificity of ELISA and immunoblot for the detection of T. solium IgG antibodies - As demonstrated in Fig. 3, all samples were tested by ELISA using the three antigen preparations. Thirty-four (85%) of the serum samples from Group 1 were positive for *T. solium* IgG antibodies in the J$_{unbound}$ fraction, 37 (92.5%) in the DJ$_{unbound}$ fraction and 33 (82.5%) in the AJ$_{unbound}$ fraction.

In Group 2, 13 (21%) of the serum samples were positive for *T. solium* IgG antibodies in the J$_{unbound}$ fraction, 14 (22.6%) in the AJ$_{unbound}$ fraction and only six (9.7%) in the DJ$_{unbound}$ fraction. As shown in Table, cross-reactivity by ELISA for the serum samples in Group 2 was predominantly due to *E. granulosus* infection. All serum samples from Group 3 were negative for *T. solium* IgG antibodies in the DJ$_{unbound}$ fraction. One (3.3%) sample from this group tested positive for *T. solium* IgG antibodies in the J$_{unbound}$ fraction and two (6.7%) samples were positive in the AJ$_{unbound}$ fraction (Fig. 3). In Groups 2 and 3, the ELISA revealed *T. solium* specificities of 84.8% for J$_{unbound}$, 93.5% for DJ$_{unbound}$ and 82.6% for AJ$_{unbound}$. These data resulted in YI values of 0.70, 0.86 and 0.65 for the J$_{unbound}$, DJ$_{unbound}$ and AJ$_{unbound}$ fractions, respectively. The DE was

determined to be 84.8%, 93.2% and 82.6% for the J$_{unbound}$, DJ$_{unbound}$ and AJ$_{unbound}$ fractions, respectively.

The immunoblot assays revealed the presence of a wide range of protein components in J$_{unbound}$, which varied in size from 24-110 kDa, as shown in Fig. 4. When using the DJ$_{unbound}$ fraction, we found that all serum samples from the patients in Group 1, who were diagnosed
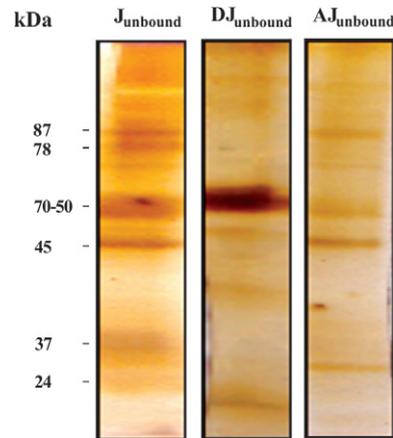

Fig. 2: electrophoretic profile of jacalin-unbound (J$_{unbound}$) fraction from *Taenia solium* metacestodes and detergent (DJ$_{unbound}$) and aqueous (AJ$_{unbound}$) phases from this fraction obtained by partitioning with Triton X-114 in sodium dodecyl sulfate polyacrylamide gel electrophoresis at 12% by silver staining. Molecular mass standards are given on the left in kDa.
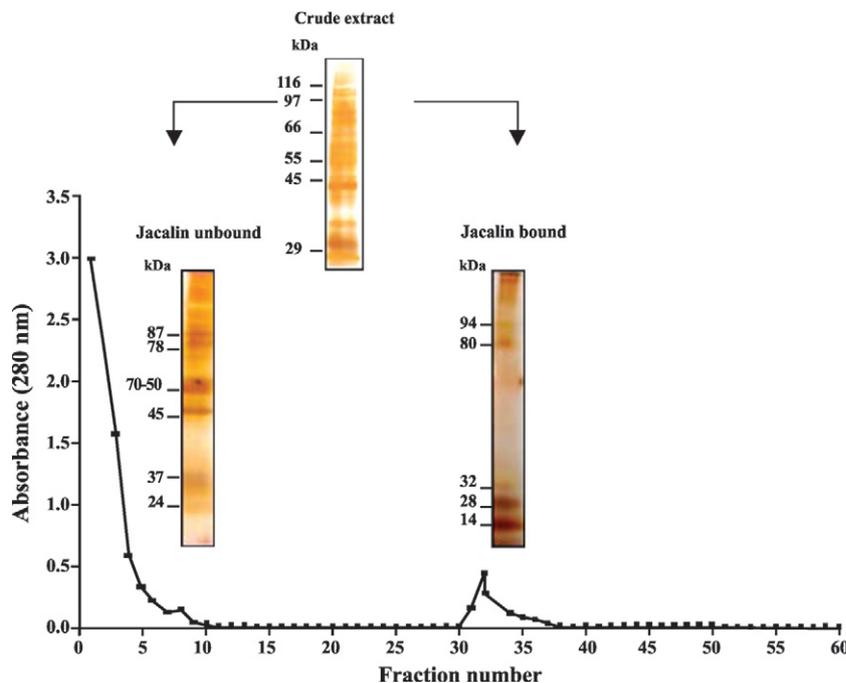

Fig. 1: affinity chromatography of saline extract of *Taenia solium* metacestodes in column of jacalin and electrophoretic profile of saline extract, jacalin-unbound and jacalin-bound in sodium dodecyl sulfate polyacrylamide gel electrophoresis at 12% by silver staining. Molecular mass standards for saline extract and molecular mass are given on the left in kDa.

with definitive NCC, reacted strongly with the 50-70 kDa component (Fig. 4A), whereas no reactivity was found for this component in the samples from Groups 2 and 3 (Fig. 4B, C). Poor reactivity was observed for all groups of serum samples using the $AJ_{unbound}$ fraction, with the predominant reactivity occurring against the high molecular weight components (Fig. 4A-C).

DISCUSSION

In the present paper, we describe the partitioning of saline extracts from *T. solium* metacestodes using jacalin affinity chromatography followed by fractionation with TX-114, demonstrating for the first time that the D phase from the $J_{unbound}$ fraction contains the antigenic components necessary for an accurate diagnosis of human NCC.

Purification by affinity chromatography resulted in $J_{unbound}$ and jacalin-bound fractions. Distinct electrophoretic profiles were observed for these two fractions; the $J_{unbound}$ fraction contained more components than the jacalin-bound fraction. The antigenic components with affinity to jacalin showed a fewer number of bands when compared to *T. solium* saline extract, but these components failed to provide sufficient information for the diagnosis of NCC.

The procedure that we describe in this paper, which utilises TX-114 phase partitioning, is fast, reproducible and relatively easy to perform because it does not require any specialised apparatuses or ultracentrifugation steps. The major advantages of using antigenic fractions purified with TX-114 are low production cost, simplicity and ease of extraction, high efficiency, speed and low toxicity from the lack of organic solvents required (Machado et al. 2007).

TABLE

Reactivity of serum samples from patients with other parasitoses (Group 2, n = 62) for detection of IgG to jacalin-unbound ($J_{unbound}$) fraction, detergent ($DJ_{unbound}$) and aqueous ($AJ_{unbound}$) phases from $J_{unbound}$ fraction from *Taenia solium* metacestodes by ELISA

	Antibody detection		
	Positive samples n (%)		
Infection (n)	$J_{unbound}$	$DJ_{unbound}$	$AJ_{unbound}$
Ascaris lumbricoides (6)	1 (16.7)	0 (0)	0 (0)
Echinococcus granulosus (10)	10 (100)	6 (60)	9 (90)
Enterobius vermicularis (6)	0 (0)	0 (0)	1 (16.7)
Giardia lamblia (4)	0 (0)	0 (0)	1 (25)
Hookworm (6)	0 (0)	0 (0)	0 (0)
Hymenolepis nana (4)	0 (0)	0 (0)	2 (50)
Schistosoma mansoni (10)	0 (0)	0 (0)	0 (0)
Strongyloides stercoralis (4)	1 (25)	0 (0)	1 (25)
Taenia sp (10)	1 (10)	0 (0)	0 (0)
Trichuris trichiura (2)	0 (0)	0 (0)	0 (0)

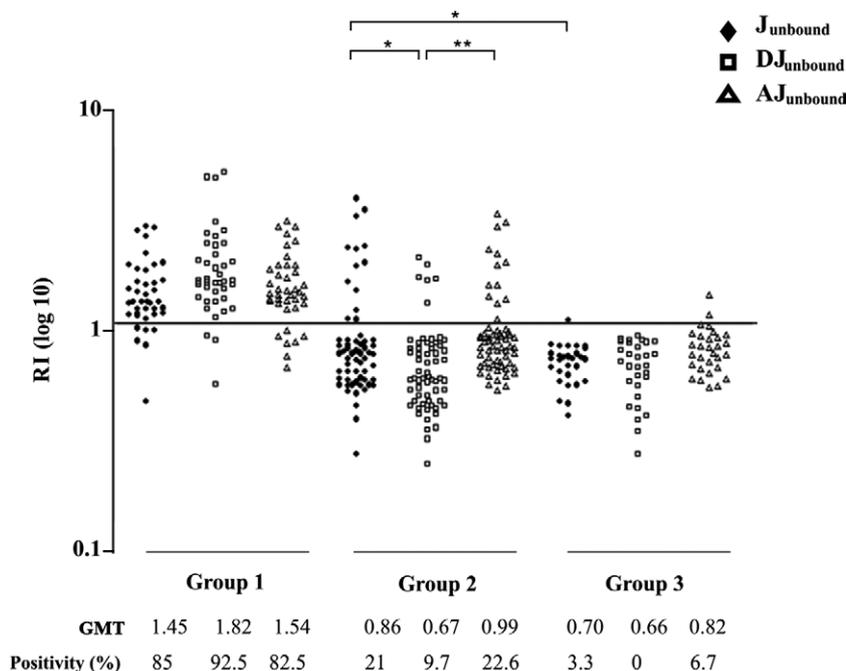|	Group 1			Group 2			Group 3		
GMT	1.45	1.82	1.54	0.86	0.67	0.99	0.70	0.66	0.82
Positivity (%)	85	92.5	82.5	21	9.7	22.6	3.3	0	6.7

Fig. 3: detection of IgG antibodies anti-*Taenia solium* metacestodes in serum samples from patients with a definitive diagnosis of neurocysticercosis (Group 1; n = 40), other parasitoses (Group 2; n = 62) and apparently healthy individuals (Group 3; n = 30) by ELISA using the jacalin-unbound ($J_{unbound}$) fraction from *T. solium* metacestodes and detergent ($DJ_{unbound}$) and aqueous ($AJ_{unbound}$) phases of this fraction. The horizontal line indicates the cut-off [reactivity index (RI) > 1.1]. GMT: geometric means of titres; *: p < 0.05; **: p < 0.005.

In the present study, jacalin affinity chromatography followed by TX-114 treatment proved to be adequate for fractionating the saline extract. When tested by ELISA, although 60% of the serum samples from patients with *E. granulosus* infections were cross-reactive, the DJ$_{unbound}$ fraction was more positive for *T. solium* IgG antibodies than the other fractions, as none of the samples from healthy individuals or those presenting other parasitoses exhibited any reactivity, indicating a significant YI and DE. The group of patients with other parasitoses is expected to represent the general population, particularly in developing countries where parasitic diseases are highly prevalent. Therefore, cross-reactivity may occur in screening tests such as ELISA, whose results would require further confirmation using an alternative immunoassay with more specific antigens.

When serum samples from patients with both NCC or *E. granulosus* infection were tested by the immunoblot assays performed on the J$_{unbound}$ fraction, it was observed a reactivity pattern that included many bands, which contributes to the complication of a differential diagnosis. In contrast, when using the DJ$_{unbound}$ fraction in the immunoblot assay, six serum samples from *E. granulosus*-infected patients did not show any reactivity for *T. solium*

IgGs and were therefore considered negative, although all of them had been considered positive by ELISA when using this antigenic fraction. Thus, the use of the DJ$_{unbound}$ fraction in immunoblot assays allows for a differential diagnosis between NCC and *E. granulosus* infection. This is a particularly significant finding because these two types of infections constitute a major cause of laboratory diagnostic ambiguity due to their cross-reactivity in various immunoassays. The 50-70 kDa band is an important component in the diagnosis of NCC. In fact, the proteins with a molecular weight of 50 kDa have been reported previously, indicating high levels of sensitivity and specificity to the diagnosis of NCC (Hancock et al. 2004, Barcelos et al. 2007, Machado et al. 2007).

The DJ$_{unbound}$ fraction showed 92.5% sensitivity and 93.5% specificity for *T. solium* IgGs, which are similar to the values reported by other authors who have used different techniques of antigen purification for NCC diagnosis (Pardini et al. 2002, Hancock et al. 2006, Machado et al. 2007). Moreover, the DJ$_{unbound}$ fraction provided superior results to those obtained by other authors using whole extracts, where the sensitivity ranged from 70-85% and the specificity ranged from 80-88% (Bueno et al. 2000, Shiguekawa et al. 2000) for the detection of
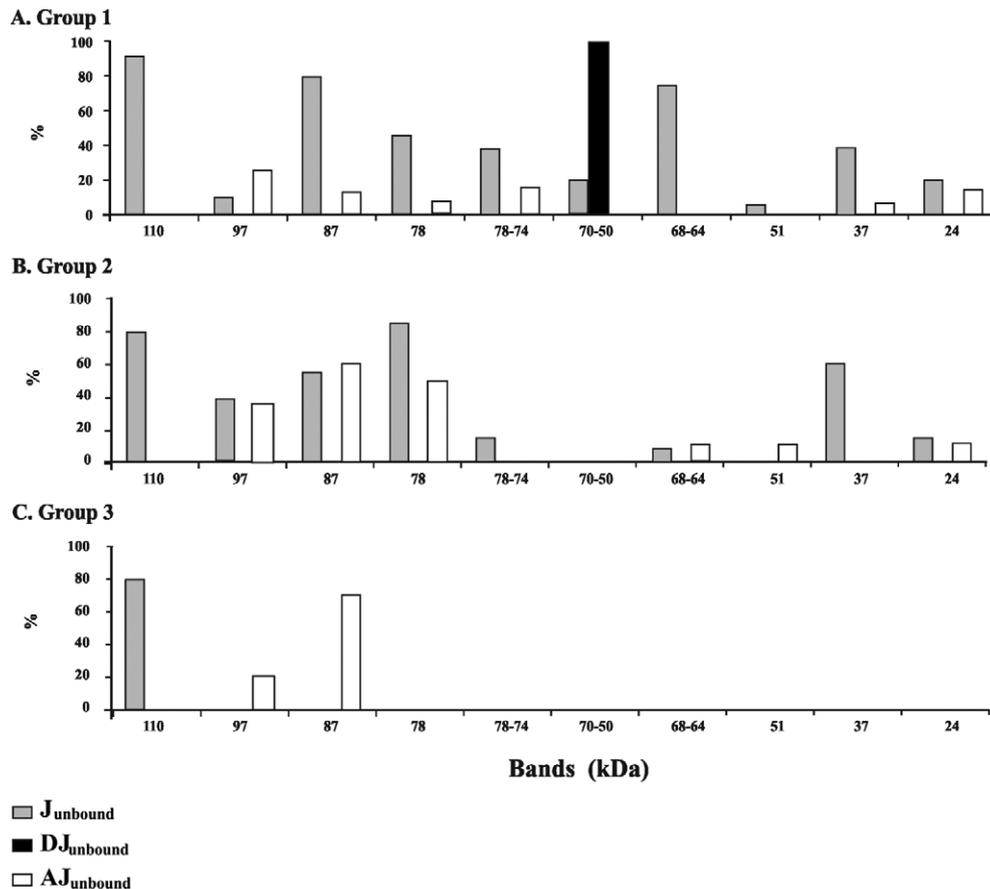

Fig. 4: percentage of reactivity frequency of serum samples ELISA reactive by immunoblot using jacalin-unbound (J$_{unbound}$) fraction from *Taenia solium* metacestodes and detergent (DJ$_{unbound}$) and aqueous (AJ$_{unbound}$) of this fraction. A (Group 1): patients with definitive diagnosis of neurocysticercosis; B (Group 2): patients with other parasitoses; C (Group 3): apparently healthy individuals.

anti-*T. solium* metacestodes IgG by ELISA. Although saline extracts have been widely used for primary screening in endemic areas, the identification and purification of highly specific glycoproteins associated with *T. solium* metacestodes have been the main focus for the serodiagnosis of NCC (Ito 2002, Lee et al. 2011). It is important to make clear that the other investigators used panels of lectins in order to identify NCC antigens and the jacalin was included in those panels (Trindade et al. 2006, Zhang et al. 2006). However, there is no previous study proposing the strategy to perform a jacalin affinity chromatography method and subsequent fractionating process by carry out a TX-114 partitioning from jacalin unbound fraction in the diagnosis of human NCC. Our results, however, demonstrate that a better diagnosis is obtained using the $DJ_{unbound}$ fraction, indicating that if glycosylated antigens are present, they should not present galactosyl residues, as the isolated components do not show affinity for jacalin, a glycoprotein known to bind only to O-glycosidically linked oligosaccharides and to prefer to bind to galactosyl (b-1,3) N-acetylgalactosamine.

Considering the immunoassay design described here and the advantages of using the $DJ_{unbound}$ fraction as the diagnostic component in immunoblot assays, it can be assumed that this assay can also be efficiently used to identify individuals with residual antibodies, as the presence of calcified metacestodes is common in endemic areas. The 50-70 kDa band visualised in the D phase by immunoblot may improve the specificity of the tests, especially in serum samples, avoiding cross-reactivity and nonspecific reactions (Ishida et al. 2003).

In conclusion, the results obtained by immunoblot confirm that unbound antigenic fractions purified from *T. solium* metacestodes by jacalin chromatography followed by TX-114 treatment, especially $DJ_{unbound}$, are important putative sources of specific antigens for diagnostic kits and have a high value for the differential immunodiagnosis of NCC.

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