Hydrophobic fraction of *Taenia saginata* metacestodes, rather than hydrophilic fraction, contains immunodominant markers for diagnosing human neurocysticercosis

Fração hidrofóbica de metacestódios de *Taenia saginata*, ao contrário da fração hidrofilica, contém marcadores imunodominantes para o diagnóstico de neurocisticercose humana

Flávia de Assunção Gonçalves¹, Gleyce Alves Machado¹,², Heliana Batista Oliveira¹,², Maria Teresa Nunes Pacheco Rezende¹, José Roberto Mineo¹ and Julia Maria Costa-Cruz²

**ABSTRACT**

**Introduction:** Considering that alternative antigens for diagnosing neurocysticercosis continue to be a challenge because of the increasing difficulty in obtaining parasites from naturally infected pigs for preparation of *Taenia solium* homologous antigen, the aim of the present study was to evaluate the detergent (D) and aqueous (A) fractions from saline extract of *Taenia saginata* metacestodes for diagnosing neurocysticercosis. **Methods:** *Taenia saginata* was obtained from naturally infected bovines in the Triângulo Mineiro region, State of Minas Gerais, Brazil. The carcasses came from cold storage units and had been slaughtered in accordance with the inspection technique recommended by the Federal Inspection Service. The D and A fractions were obtained by using Triton X-114 (TX-114). Serum samples were obtained from 40 patients with a diagnosis of neurocysticercosis, 45 with other parasitic diseases and 30 from apparently normal individuals. IgG antibody levels were evaluated using the ELISA and immunoblotting assays. **Results:** The ELISA sensitivity and specificity were 95% and 73.3%, when using saline extract; 95% and 82.6% for the D fraction; and 65% and 61.3% for the A fraction, respectively. The immunoblotting assay confirmed the ELISA results, such that the D fraction was more efficient than the other extracts, and the 70-68kDa component was immunodominant among neurocysticercosis patients. **Conclusions:** These results demonstrated that the D fraction from *Taenia saginata* metacestodes obtained using TX-114 can be used as a heterologous antigenic fraction in the immunoblotting assay for serologically diagnosing human neurocysticercosis, given its ability to select immunodominant antigens.


**RESUMO**

**Introdução:** Considerando que antígenos alternativos para o diagnóstico da neurocisticercose (NC) continuam sendo um desafio devido ao aumento da dificuldade em se obter parasitas de suínos naturalmente infectados, para a preparação do antígeno homólogo de *Taenia solium*, o objetivo do presente estudo foi avaliar frações detergente (D) e aquosa (A), do extrato salino de metacestódeos de *Taenia saginata* para diagnóstico de NC. **Métodos:** Bovinos, naturalmente infectados com *Taenia saginata*, procedentes da região do Triângulo Mineiro, Estado de Minas Gerais, Brasil, foram obtidos de frigoríficos e abatidos de acordo com a técnica de inspeção recomendada pelo Serviço de Inspeção Federal. As frações D e A foram obtidas utilizando Triton X-114 (TX-114). Amostras de soro foram obtidas de 40 pacientes com diagnóstico de neurocisticercose, 45 com outras doenças parasitárias e 30 de indivíduos aparentemente normais. Níveis de IgG foram avaliados pelos testes ELISA e Imunoblotting. **Resultados:** A sensibilidade e especificidade do teste ELISA foram 95% e 73,3%, quando utilizado o extrato salino, 95% e 82,6% para a fração D, e 65% e 61,3% para a fração A, respectivamente. O ensaio Imunoblotting confirmou os resultados do teste ELISA, sendo a fração D mais eficiente que os outros extratos, observando-se que o componente 70-68kDa se comportou como imunodominante para os pacientes com NC. **Conclusões:** Estes resultados demonstraram que a fração D de metacestódeos de *Taenia saginata* obtida com TX-114 pode ser utilizada como fração antigênica heteróloga pelo Imunoblotting para o diagnóstico sorológico da NC humana, considerando sua habilidade para selecionar antígenos imunodominantes.


**INTRODUCTION**

Neurocysticercosis (NC) is the most common infection of the human central nervous system and it is caused by the larval stage of the intestinal parasite *Taenia solium*. This disease affects millions of people worldwide, especially in areas with poor sanitation where humans and animals live in close contact, and in regions where meat inspection is lax¹.

The major clinical manifestations of NC are seizures, intracranial hypertension and focal neurological deficits. Sequelae such as epilepsy, hydrocephalus and dementia can also occur²,³. Neuroimaging techniques have contributed towards greater diagnostic accuracy. Only the presence of cystic lesions showing the scolex has been considered pathognomonic⁴,⁵. These techniques are expensive and generally inaccessible in endemic rural situations. Consequently, various assays have been developed for detecting anticysticercal antibodies in serum, saliva and cerebrospinal fluids (CSF)⁶-⁹.

Different antigenic extracts from *Taenia solium* metacestodes, such as saline extract, vesicle fluid, alkaline extract, scolex or membrane extracts have been used in the enzyme-linked immunosorbent assay (ELISA) for NC diagnosis and have shown differences in terms of sensitivity and specificity¹⁰-¹². The immunoblotting (IB) assay has also been used for diagnosing NC, and different indices of sensitivity and specificity have been observed, depending on the antigen preparation used¹³-¹⁵.

Alternative antigens for NC immunodiagnosis continue to be a challenge because of the increasing difficulty in obtaining parasites from naturally infected pigs for preparation of *Taenia solium* homologous antigen¹⁶. Heterologous antigens from *Taenia saginata* have also been used with satisfactory results in ELISA and IB, and can be used as alternative antigens for diagnosing human NC¹⁷-¹⁹. *Taenia saginata* cysticerci in their cystic stage can be obtained in considerable quantities,
because the habitual diet in many countries is mainly based on beef consumption. Although heterologous antigens from Taenia saginata metacestodes have been used with satisfactory results, there has been no research to test purification of these antigens with the aim of selecting antigens present in this helminth that are able to react with antibodies induced by Taenia solium-infected individuals. Cross-reactivity has been demonstrated among helminths, particularly between Taenia and Echinococcus, which share the same antigenic components. Therefore, it is necessary to carry out procedures to select immunogenic components from saline extracts of Taenia saginata that are homologous to Taenia solium, but not shared by other helminths. Even though there have been previous reports on the advantages of recombinant antigens or synthetic peptides from Taenia solium and Taenia saginata, it can be demonstrated that native antigens present better diagnostic performance, possibly due to post-translational modifications such as glycosylation pathways that are not present in the prokaryotic system.

The procedure using Triton X-114 (TX-114) fraction partitioning is a versatile and efficient purification technique. A solution of TX-114 nonionic surfactant is homogeneous at 0°C but separates into detergent (D) and aqueous (A) fractions above 20°C. Integral membrane proteins of an amphiphilic nature are recovered in the D fraction and hydrophilic proteins are found exclusively in the A fraction. Antigenic fractions purified from Taenia solium metacestodes by means of TX-114, especially the D fraction, have been shown to be efficient for diagnosing NC.

The aim of the present study was to evaluate hydrophobic and hydrophilic fractions from Taenia saginata metacestodes obtained by means of TX-114 extraction for immunodiagnosis of human NC.

**METHODS**

**Patients and serum samples**

Serum samples were collected from 115 subjects selected by the Clinical Analysis Laboratory of the Clinical Hospital (Groups 1 and 2) and from the Parasitology Laboratory (Group 3) of the Federal University of Uberlândia, in the State of Minas Gerais, Brazil. Group 1 was formed by 40 patients who had been diagnosed with definitive NC, based on the presence of the clinical syndrome, evidence of the parasite by neuroimaging, epidemiological data and positive immunological tests, according to diagnostic criteria defined previously. All the patients presented at least one type of clinical manifestation suggestive of NC such as: epilepsy (55%), headache (50%), dizziness (27.5%), dementia (12.5%), faintness (10%) or hydrocephalus (2.5%), and no signs or symptoms of cysticercosis in other organs. They presented evidence of parasite neuroimaging, with the following characteristics: 8 (20%) vesicular; 15 (37.5%) vesicular/calcified; and 17 (42.5%) calcified metacestodes. All the patients came from or lived in an area where cysticercosis is endemic. At least two patients had had household contact with Taenia solium infection and they were tested positive using cerebrospinal fluid ELISA for detection of IgG anticysticercal antibodies, as previously described.

Serum samples from Group 2 were obtained from 45 patients with other parasitoses: Ascaris lumbricoides (6), Echinococcus granulosus (6), Enterobius vermicularis (5), Ancylostoma duodenale or Necator americanus (5), Hymenolepis nana (4), Schistosoma mansoni (5), Strongyloides stercoralis (4), Taenia sp (8) and Trichuris trichura (2). The Group 3 serum samples were from 30 apparently healthy volunteers, based on their clinical picture and three fecal samples that tested negative using the Lutz and Baermann parasitolological methods. All these individuals came from endemic areas for cysticercosis and they did not present any evidence of household contact with Taenia solium infection or previous history of taeniasis or cysticercosis.

**Antigen preparations**

Saline extract from Taenia saginata metacestodes was prepared as described by Oliveira et al. The D and A fractions were obtained using a solution of TX-114 (Sigma Chem Co, St Louis, MO, USA), as described by Machado et al., with modifications from the original procedure, as described by Bordier. The saline extract of Taenia saginata metacestodes (8.4mg of total protein) was added to 1,680 μl of Tris-buffered saline (10mM Tris-HCl, pH 7.4, 150mM NaCl) and 1% TX-114. This mixture was incubated (0°C, 10 min) and added carefully to a sucrose mixture (5.5ml of Tris plus 6% sucrose and 0.06% TX-114). The mixture was incubated (37°C, 10 min), followed by centrifugation (3,000g, 25°C, 10 min). The upper fraction was collected and 1% TX-114 was added and incubated (0°C, 10 min). This solution was again added carefully to the sucrose mixture used previously, incubated (37°C, 10 min) and centrifuged (3,000g, 25°C, 10 min). The supernatant was reserved for separation of the A fraction and the pellet consisted of the D fraction. The recovered supernatant was rinsed with 2% TX-114 in a separate tube without a sucrose cushion, incubated at 0°C for 10 min and then at 37°C for 10 min, followed by centrifugation (3,000g, 25°C, 10 min). The supernatant of this solution comprised the A fraction. The purified proteins (D and A fractions) were precipitated (1:2; v/v) in cold acetone at 4°C overnight and centrifuged (3,000g, 4°C, 30 min). The supernatants were discarded carefully and the precipitates were resuspended in 1ml of Tris-buffered saline. The protein content of each antigen preparation was determined in accordance with Lowry et al., using bovine serum albumin as the standard protein, and the aliquots were stored at -20°C.

**Enzyme-linked immunosorbent assay**

ELISA was carried out to detect IgG antibodies to Taenia saginata metacestodes, as previously described by Oliveira et al. Optical densities (OD) were determined at 492nm in an ELISA reader (Tp Reader, Thermostate, China). The cutoff values were established using the mean OD of three non-reactive serum samples plus two standard deviations, as described by Bassi et al. The reactive index (RI) was calculated as the ratio between optical density and cutoff, as described by Pardini et al. All the samples showing RI > 1 were considered positive.
RESULTS

SDS-polyacrylamide gel electrophoresis

The antigens were diluted (v/v) in sample buffer and, after boiling at 100°C for 3 min, all antigens and molecular weight markers (Sigma) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 12% under non-reducing conditions, as previously described by Laemmli. The antigen bands and molecular weight markers were viewed by means of silver staining, as described by Friedman, and the relative molecular masses were estimated using a logarithmic plot of the migration of the molecular weight standards included in each gel.

Immunoblotting

Antigen preparations were subjected to electrophoresis and transferred to nitrocellulose membranes (0.45mm, Sigma), as previously described by Towbin et al, by using a transfer apparatus (Omniphor, England). The IB assay was performed as described by Oliveira et al, by using serum samples diluted at 1:100 and peroxidase-labeled goat anti-human IgG conjugate whole molecule (Sigma) diluted 1:1,500 in 1% PBS-TM. The strips were developed for 3 min in substrate solution containing hydrogen peroxide and 3,3-diaminobenzidine tetrahydrochloride (DAB-Sigma) in PBS. The reaction was stopped with distilled water and reactions were considered positive if clearly defined bands appeared. The relative molecular masses of the recognized bands were determined by comparison with molecular markers (Sigma).

Data analysis

The geometric means (gm) of RI were calculated for each subject group and antigen extract. The difference between pairs of proportions was calculated by means of a binomial test (Z statistics). The data were analyzed using BioStat 2.0 software. The null hypothesis was rejected when p < 0.05. Sensitivity, specificity and diagnostic efficiency (DE) were calculated in accordance with Barbieri et al, and the Youden Index (YI) was determined in accordance with Youden.

Ethical

This study was approved by the Ethics Committee of the Federal University of Uberlândia.

Enzyme-linked immunosorbent assay

All samples were tested by means of ELISA using the three antigen preparations (Figure 1). In Group 1, statistical differences were found between the saline extract and A fraction, and between the D and A fractions (p < 0.05). In Group 2, a statistical difference was found only between the D and A fractions (p < 0.05). No statistical difference was observed in Group 3. The sensitivity for detection of IgG antibodies to Taenia saginata was 95% for both saline extract and D fraction antigens and 65% for A fraction. In Group 2, 42.2% and 64.4% of the serum samples were positive for saline extract and A fraction antigens, respectively. Only 28.9% of the serum samples of this group were tested positive for D fraction. Cross-reactivity in ELISA for Group 2 was predominantly found in serum samples of patients with Echinococcus granulosus, Ascaris lumbricoides and Hymenolepis nana (Table 1). All serum samples from Group 3 were negative for both D and A fractions, while 3.3% of the samples

<table>
<thead>
<tr>
<th>Parasites</th>
<th>Number of serum samples examined</th>
<th>Saline n+</th>
<th>%</th>
<th>D phase n+</th>
<th>%</th>
<th>A phase n+</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascaris lumbricoides</td>
<td>6</td>
<td>5</td>
<td>83.3</td>
<td>4</td>
<td>66.7</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>Echinococcus granulosus</td>
<td>6</td>
<td>6</td>
<td>100</td>
<td>6</td>
<td>100</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>Enterobius vermicularis</td>
<td>5</td>
<td>2</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Hymenolepis nana</td>
<td>4</td>
<td>3</td>
<td>75</td>
<td>3</td>
<td>75</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Anoplopla duodenale or Necator americanus</td>
<td>5</td>
<td>2</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Schistosoma mansoni</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Strongyloides stercolalis</td>
<td>4</td>
<td>1</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Taenia sp</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

n+: positive samples.
from this group tested positive for the saline extract antigen. ELISA specificity was 73.3% (saline extract), 82.6% (D fraction) and 61.3% (A fraction), whereas the diagnostic efficiency and Youden index were 83.9% and 0.74 (saline extract), 88.5% and 0.80 (D fraction), and 72.8% and 0.46 (A fraction), respectively.

Electrophoresis of saline extract and fractionated antigens
As demonstrated in Figure 2A, the electrophoretic profile of the saline extract and A fraction antigens from Taenia saginata metacestodes in silver-stained SDS-PAGE showed several bands, while the D fraction showed four clearly defined components with apparent molecular sizes of 70, 64, 50 and 39kDa.

Immunoblotting
A representative IB is demonstrated in Figure 2B. The immunoreactive antigenic bands recognized from serum samples of patients in Groups 1 and 2 varied from 116 to 24kDa for the saline extract and D fraction antigens and 116 to 13kDa when using the A fraction (Figure 3). When using the D fraction, it was found that patients with a definitive diagnosis of NC reacted strongly with the 70-68kDa components (Figure 3A). On the other hand, patients with Echinococcus granulosus infection showed no reactivity with the 70-68kDa components, even though the presence of various reactive components was observed (116, 110, 97, 87, 64, 45, 42-39 and 24kDa) (Figure 3B). For the A fraction, reactivity was observed mostly for high molecular weight components (116 and 110kDa).

DISCUSSION
The use of heterologous antigens to diagnose human NC has been described mainly in relation to Taenia crassiceps antigen, in both serum and CSF samples. Furthermore, high sensitivity in ELISA and IB tests when using saline extract obtained from Taenia saginata metacestodes has already been demonstrated.

In the present study, we demonstrated for the first time in the literature that partitioning of saline extract from Taenia saginata metacestodes into two fractions, a detergent (D) and an aqueous (A) fraction, using TX-114 solution, is a useful procedure for diagnosing human NC. The major advantages of using antigens purified by TX-114 solution are the minimal cost of producing them in the laboratory, simple and easy extraction, high efficiency, speed and lower toxicity to the environment than from extractions involving organic solvents. Satisfactory results have been obtained using D fraction antigens, in which the performance of integral membrane antigens recovered from the D fraction during TX-114 treatment has shown their promise as candidate antigens in ELISA-based serodiagnosis, with substantial sensitivity, specificity and safety.

In the present study, the D fraction was shown to be more specific than the other extracts, while none of the serum samples from healthy individuals were reactive. The diagnostic efficiency and Youden index confirmed the good performance by ELISA, using the D fraction from Taenia saginata metacestodes for diagnosing...
human NC. Similar results have been obtained from partitioning of saline extract from *Taenia solium* metacestodes\(^{14}\).

Because of the lower sensitivity and specificity of the A fraction obtained from *Taenia saginata* metacestodes, it is not useful for detecting anti-*Taenia solium* metacestode IgG antibodies for serologically diagnosing human NC. Similar results have been obtained with the aqueous fraction from *Taenia solium* metacestodes\(^{14}\).

In the present study, when using the D fraction purified with TX-114, cross-reactivity in ELISA occurred mainly in serum samples from patients with *Echinococcus granulosus*, *Ascaris lumbricoides* and *Hymenolepis nana*. On the other hand, when using the A fraction, cross-reactivity was observed for the majority of the serum samples from patients with other parasites, except for *Taenia* sp and *Trichuris trichiura*. These results suggest that ELISA can be used as a screening test, whereas IB is a confirmatory assay.

Previous study had demonstrated that *Taenia* sp metacestode antigens were likely to be stage specific, since there was high sensitivity with serum samples from individuals infected with metacestodes and high specificity in individuals with *Taenia* sp infection\(^{14}\).

The presence of shared antigenic components between *Taenia* sp and *Echinococcus* sp, which belong to the same family (*Taeniidae*), has been demonstrated and these antigens are probable responsible for the cross-reactivity between these parasites\(^{16}\). Seven immunodominant proteins (135, 100, 86, 64, 39, 35 and 24kDa) had already been recognized in serum samples from individuals infected with *Echinococcus granulosus*, in IB using vesicle liquid from *Taenia solium* metacestodes\(^{17}\). In the present study, various proteins from saline antigen were also recognized in patients infected with *Echinococcus granulosus*, as demonstrated previously by our group\(^{18}\) in relation to using *Taenia saginata* metacestodes. In addition, cross-reactivity among individuals infected with *Hymenolepis nana* was observed, which had been shown in a previous study\(^{19}\). This is related to the phylogenetic proximity between *Taenia* sp and *Hymenolepis* sp (*Taeniidae* family) and to the presence of shared antigenic components between these species\(^{18}\).

In the present study, the higher sensitivity and specificity of IB using the D fraction was related to the expression of the 70-68kDa band, which was considered to be an immunodominant component in diagnosing NC patients by means of *Taenia saginata* metacestodes. Accordingly, components of 70-50kDa have been already reported in our previous study using the D fraction from *Taenia solium* metacestodes, showing satisfactory sensitivity and specificity levels\(^{24}\). Thus, future studies, particularly those to be carried out under field conditions, should consider the possibility of using purified 70-68kDa components in easy-to-perform tests, such as immunodot assays.

In conclusion, the results presented here demonstrated that the D fraction from *Taenia saginata* metacestodes obtained using TX-114 can be used as heterologous antigenic fraction in immunoblotting for serologically diagnosing human NC, given its ability to select immunodominant antigens.

---

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

---

**FINANCIAL SUPPORT**

CNPq, CAPES, FAPEMIG.

**REFERENCES**


