SIMILAR DIAGNOSTIC PERFORMANCE FOR NEUROCYSTICERCOSIS OF THREE GLYCOPROTEIN PREPARATIONS FROM TAENIA SOLIUM METACESTODES

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Abstract. The detection of antibodies to Taenia solium metacestodes is very important in the differential diagnosis of neurocysticercosis (NCC). In this study, an electroimmunotransfer blot (EITB) assay that uses an elaborate protocol with metacestode glycoproteins as antigens was compared with two other Western blots that use glycoproteins obtained using simpler methods, including an eluate from a lectin column, or the vesicular fluid (VF) of the parasite. The concordance between the three assays was 91% in patients with active NCC and 100% in patients with suspected NCC and previous documentation of negative serology. The specificities for the Western blots and the EITB assay were 98% and 100%, respectively (98% concordance). These data suggest that the simplest of these immunosassays, the one that uses the VF of T. solium metacestodes in a Western blot format, can be reliably used for the serologic diagnosis of NCC in developing countries where access to the EITB assay is difficult.

INTRODUCTION

Infection of the human brain with Taenia solium metacestodes or cysticerci is known as neurocysticercosis (NCC). This parasitic disease can cause a variety of neurologic abnormalities, with the most common being seizures and signs and symptoms of increased intracerebral pressure (papilledema, headache, vomiting). In the clinical setting, the diagnosis of NCC is not simple because similar clinical and radiologic findings can also be present in other diseases of the central nervous system (CNS). In fact, the only way of obtaining a definitive diagnosis is through surgical removal and subsequent identification of the parasite. However, this invasive procedure is seldom performed. Therefore, a diagnosis of NCC is usually obtained after combining the clinical findings with the radiologic, serologic, and epidemiologic data. The detection of antibodies against cysticercal antigens in the blood or cerebrospinal fluid is of major importance in the diagnosis of NCC. The best characterized immunoassay for diagnosis of NCC is the electroimmunotransfer blot (EITB) assay, which detects antibodies to putative glycoprotein antigens of the metacestode. However, this assay is expensive and therefore not readily available for patients of a low socioeconomic status who are infected with this parasite. Furthermore, the procedure for the preparation of the antigen for the EITB assay is complex and requires sophisticated technology. For this reason, investigators have continued to search for simpler and more practical alternatives for the serologic diagnosis of NCC. Some groups have reported the production of recombinant antigens from the cysticercus that appear to be promising. Other investigators have used semi-crude preparations of T. solium or T. crassiceps metacestodes, with the most common being the vesicular fluid (VF) of these parasites. The results with VF indicate that this preparation is highly antigenic, but it has variable sensitivity and specificity when used with sera in enzyme-linked immunosorbent assays (ELISAs).

We previously reported a simple method for isolating T. solium metacestode glycoprotein antigens by passing the aqueous phase of the parasite extract through a Lens culinaris affinity column. The proteins in the eluate were then resolved under reducing conditions by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and antibodies to the 12-, 16-, 18-, 24-, and 28-kD glycoprotein antigens were detected by Western blotting (eluate-WB) in sera from patients with NCC. In this initial evaluation of the eluate-WB, patients with NCC could be distinguished from those with other parasitic diseases, including echinococcosis. Cloning and sequencing of two genes showed that at least two of these glycoproteins could also be detected in the EITB assay: the 12-kD antigen (14-kD in the report by Greene and others) and the 18-kD antigen (GenBank accession numbers AF257776, AF350070, AFO082829, and AFO082828).

In the present study, we found that the VF of T. solium cysticerci was rich in the glycoprotein antigens used in the eluate-WB. Following up on this observation, we compared the performance of the eluate-WB, the VF Western blot (VF-WB), and the commercial version of the EITB assay (Immunetics, Cambridge, MA) using a collection of sera from patients with either active (confirmed or probable) or suspected NCC, and from healthy individuals or patients with heterologous parasitic infections or CNS pathologies as specificity controls.

MATERIALS AND METHODS

Parasites and antigen preparation. Taenia solium cysticerci were dissected from naturally infected pigs and used to prepare a glycoprotein-enriched fraction exactly as previously described. Briefly, the cysticerci were homogenized in a cocktail of proteinase inhibitors (5 mM phenylmethylsulfonyl fluoride, 0.3 μM aprotinin, 0.1 μM leupeptin, and 0.1 μM pepstatin; Sigma), passed through an H. encephalitis affinity column, and the N-linked glycoproteins were eluted with 0.2 M D-mannoside (Sigma, St. Louis, MO). Another antigen source was the glycoprotein-rich VF that was aspirated from each cysticercus with a tuberculin syringe, and then mixed with the cocktail of proteinase inhibitors described above. The two antigen preparations were stored in aliquots at −20°C until used.

Analysis of glycoprotein antigens and immunoassays. The eluate and VF preparations were separated on a mini-protein II (BioRad, Hercules, CA) preparative 15% SDS-PAGE gel.
(7.3 × 10.2 cm) under reducing conditions for subsequent silver staining and Western or lectin blots. For Western blotting, the proteins were transferred onto a nitrocellulose membrane (Trans-Blot Transfer Medium; BioRad) and individual strips (4 mm) were then cut and stored at −20°C until used. When needed, the strips were thawed and used for Western blot analysis with the serum from patients with NCC and controls at a dilution of 1:200. Positive (NCC confirmed) and negative (healthy) controls were included to ensure that an appropriate amount of antigen was present in the nitrocellulose membrane resulting from each preparative gel. The commercial Qualicode Cysticercosis kit was used as described by the manufacturer (Immunetics, Cambridge, MA). The strips containing the eluate, VF, and EITB assay antigens were also used for lectin blots with biotinylated L. culinaris or concanavalin A (ConA) as previously described. Kappa values to establish concordance between the assays were obtained using SPSS version 8.0 software (SPSS, Inc., Chicago, IL).

**Patient specimens.** A retrospective analysis of our collection of serum specimens was performed. Based on the medical records, the sera from three groups of patients were selected. The first group was composed of 44 patients with active NCC. Eight were biopsy confirmed, and 36 had probable NCC because they presented neurologic symptoms, living in a region endemic for NCC (Colombia or Mexico), and had a medical history of positive serology for NCC by different methods, including ELISA, eluate-WB, or the commercial version of the EITB assay (Qualicode Cysticercosis; Immunetics). Thirty-one had radiologic findings compatible with NCC. Information was not available for 13 patients. The second group of patients consisted of 18 individuals classified as having suspected NCC because they had clinical, epidemiologic, and radiologic data compatible with NCC, but negative serologic results in their medical records. Radiologic data indicated the presence of brain lesions that were calcified (14 cases), cystic (2 cases), hypodense (1 case), or intraventricular (1 case). The third group consisted of 55 specificity controls. Twenty-four were healthy, non-symptomatic individuals from Colombia, six had other CNS pathologies (2 with chronic meningitis, 2 with brain tumors, 1 with hypoxic encephalopathy, and 1 with herpetic meningoencephalitis), and 25 had other parasitic infections that included six with ascarosis, one with giardiosis, one with combined malaria/filariaisis, and 17 with echinococcosis. Twelve of the echinococcosis specimens were kindly donated by Drs. Alberto Nieto and Gualberto Gonzalez (Universidad de la Republica Oriental del Uruguay, Montevideo, Uruguay). The study was reviewed and approved by the Ethics Committees of the Corporacion para Investigaciones Biologicas, the Hospital Universitario San Vicente de Paul, the Hospital Pablo Tobón Uribe, and the University Hospital of the University of Texas Health Science Center (San Antonio, TX).

**RESULTS**

**Characterization of the antigens in the eluate-WB, VF-WB, and EITB assay.** The isolation of antigenic T. solium glycoproteins by preparing a cysticercus homogenate, passing it through a L. culinaris affinity column, and analyzing the glycoproteins by Western blot (eluate-WB) to detect antibodies directed against them is a relatively simple method when compared with the more elaborate protocol for performing the EITB assay. In this study, the serendipitous observation that there is an approximately 1,000-fold improvement in the yield of antigenic glycoproteins by using the VF directly suggested that this latter approach could further simplify the preparation of glycoprotein-based antigens for NCC serology. To explore this possibility, the components of the eluate antigen and the VF of the parasite were compared by silver staining, Western blotting with NCC sera, and lectin blots with biotinylated L. culinaris and ConA. Silver staining and Western blotting (Figure 1) indicated that the two preparations had similar antigens with molecular masses of 12, 16, 18, 24, and 28 kD. The results of the lectin blot were also consistent with the previously documented presence of N-linked carbohydrates attached to each of these antigens.

It was likely that the eluate and VF also shared some antigens with those in the EITB assay because these methods are based on T. solium molecules isolated on the basis of their affinity for L. culinaris. Furthermore, nucleotide sequence data indicated that the 12-kD and 18-kD antigens were subunits of the higher molecular mass multimeric antigens that are used under non-reducing conditions in the EITB assay (GenBank accession numbers AF257776, AF350070, AFO082829, and AFO082828). To further characterize the antigens of the EITB assay, their glycoprotein nature was confirmed by lectin blots using L. culinaris and ConA. However, only the 39–42-kD and 50-kD bands from the EITB assay were positive.

**Specificity and interpretation of the results of the serologic assays.** The specificities of the eluate-WB, VF-WB and EITB assay were evaluated with the sera from 24 non-symptomatic individuals from Colombia, 25 patients with heterologous parasitic infections, and six patients with other CNS patholo-

**FIGURE 1.** Antigenic composition of the eluate (Elu) and vesicular fluid (VF) of cysticerci of *Taenia solium*. These components were resolved under reducing conditions by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and subjected to **A**, Silver staining and **B**, Western blot analysis with a pool of seven sera diluted 1:200 from patients with neurocysticercosis. Both preparations have the same 12-, 16-, 18-, 24-, and 28-kD glycoprotein antigens. Molecular mass (M<sub>r</sub>) is indicated in kilodaltons (kD).
gies. The eluate-WB and VF-WB were considered positive when at least two of the 12-, 16-, 18-, 24-, and 28-kD glycoprotein antigens were detected. Based on the manufacturer’s instructions, the result of the EITB assay was positive when there were detectable antibodies against any of the 14-, 18-, 21-, 24-, 39−42-, or 50-kD bands.4 Bands larger or smaller than the target antigens in all three assays were not considered positive. Based on these parameters, the specificity of the assays was 100% for the EITB assay and 98% for the eluate WB and VF-WB. In the latter two assays, the only positive specimen was from one of the 17 patients with echinococcosis tested (Table 1).

Comparative serology with the sera of patients with active or suspected NCC. The performance of the eluate-WB, VF-WB and EITB assays was compared by testing the serum specimens from patients with active (44 cases) or suspected NCC (18 cases). The performance of the eluate-WB and VF-WB was identical. In the patients with active NCC, there was positive serology in 93% and 98% of the cases evaluated with either of the two Western blots or the EITB assay, respectively (Table 1). The EITB assay and the two Western blots showed identical results in 40 of the 44 patients (91%) in this study group. Serum samples from the four patients that showed discordant results between the assays corresponded to three faintly positive samples by the EITB assay and negative by the Western blots, and one case with the opposite results. All patients with suspected NCC had negative serology by the three assays (Table 1), a finding that is consistent with the previous serologic results in their medical records. The data from the two groups of patients indicated a concordance of 92% (kappa = 0.82, P < 0.001) between the Western blots and the EITB assay.

Further analysis of the eluate-WB and VF-WB indicated that the positive bands were also similar for a given patient, with the most commonly recognized being the 12-kD (98% and 95%, respectively), 18-kD (95% and 93%, respectively), 24-kD (85% in both assays), and 16-kD (68% in both assays) bands. The main difference between these two assays was the lower detectable reactivity of the 28-kD band in the VF-WB (35%) compared with the eluate-WB (55%). However, this difference did not affect the overall diagnostic performance of the VF-WB when compared with the eluate-WB because none of the patients had a single 28-kD positive band. The most common bands in the EITB assay had molecular masses of 24 (94%), 39−42 (82%), 50 (80%), 14 (69%), 21 (37%), and 18 kD (31%). The results for four patients with positive serology by the three assays are shown in Figure 2.

**DISCUSSION**

In the present study, the performance of the EITB assay was compared with that of two other Western blot assays (the eluate-WB and the VF-WB) that are technically simpler and also use glycoprotein antigens from *T. solium* cysticerci. Since the latter two assays gave virtually identical results with samples from patients with active or suspected NCC or with heterologous specificity controls, they are collectively referred to as Western blots. The EITB assay and the Western blots showed identical results in 91% and 100% of the patients with active or suspected NCC, respectively. The specificity was 98% for the Western blots and 100% for the EITB.

**Table 1**

<table>
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<tr>
<th>Immunoassays</th>
<th>Neurocysticercosis</th>
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<td></td>
<td>Active</td>
<td>Suspected</td>
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<tr>
<td>Eluate-WB</td>
<td>41/44 (93)</td>
<td>0/18 (0)</td>
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<tr>
<td>VF-WB</td>
<td>41/44 (93)</td>
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<tr>
<td>EITB</td>
<td>43/44 (98)</td>
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* Values are no. of positive cases/no. tested (%). WB = Western blot, VF = vesicular fluid; EITB = electroimmunotransfer blot.
† Specificity controls include heterologous parasitic infections (n = 25), CNS pathologies (n = 6), and healthy individuals (n = 24).

**Figure 2.** Western blot analysis of serum samples from patients with positive serologic results for neurocysticercosis. Four representative positive serum specimens (lanes 1–4) gave essentially the same staining pattern for the 12-, 16-, 18-, 24-, and 28-kD bands in A, the eluate Western blot (eluate-WB) and B, the vesicular fluid Western blot (VF-WB). These samples also showed the 14-, 18-, 21-, 24-, 39−42-, and 50-kD bands in the electroimmunotransfer blot (EITB) assay. Positive (+) and negative (-) controls are shown for each assay. A faint 12-kD band in the negative control (arrow in the VF-WB) was occasionally observed, but this sample is considered negative. Molecular mass (M_r) is indicated in kilodaltons (kD).
assay. The concordance between the Western blots and the EITB assay in patients with active and suspected NCC and in the specificity controls was 95% (kappa = 0.89, P < 0.001). These data indicate that there are alternative glycoprotein-based immunoassays for the diagnosis of NCC that do not require high technology. Therefore, these may be easily standardized in clinical and research laboratories in regions endemic for NCC.

The comparable results obtained with the three assays are consistent with the similar compositions of the antigens used in them. The eluate-WB and VF-WB use essentially the same five antigens. In addition, some of the multimers used in the EITB assay appeared to contain the same 12-kD (14 kD in the study by Greene and others) and 18-kD subunits found in the eluate and VF. Based on the lectin affinities analyzed in this study and on the previous multimetric bands observed under non-reducing conditions with the 12–28-kD antigens, the 12-kD and 18-kD antigens in the Western blots may be subunits of the 39–42-kD and/or 50-kD multimeric bands in the EITB assay. Nevertheless, there are subtle differences in the assays that may explain the minor differences in their performance. Since the EITB assay is conducted under non-reducing conditions, it is more likely to contain conformational epitopes that are absent in the reduced glycoproteins used in the two Western blot assays. In addition, the antigens used in the EITB assay may contain additional antigens that are not present in the eluate or VF assay. This is likely because the protocol used in conjunction with the EITB assay was designed to isolate proteins from the aqueous and non-aqueous phase of the cysticercus, while the Western blots contain only water-soluble glycoprotein antigens. The lack of affinity for L. culinaris and ConA of the 14-, 18-, 21-, and 24-kD antigens used in the EITB assay may be consistent with this observation.

In the present study, VF gave a higher yield of essentially the same antigens present in the previously described eluate. We observed that a 1:1,000 dilution of VF contained sufficient antigen to be resolved in one well by SDS-PAGE. In any country endemic for NCC, the collection and storage of this fluid at -20°C is simple. Furthermore, we observed that the native antigens were stable for at least one year when stored frozen in either a suspension of proteinase inhibitors or on nitrocellulose strips. Thus, this assay can be performed in any laboratory that is set up to do Western blot assays. A critical aspect of the VF-WB assay is to use the optimal amount of antigen to ensure consistent sensitivity and specificity. Therefore, careful titration with established positive and negative controls is essential when each preparative gel is run and transferred. Another important aspect is the interpretation of the results of the assay because some sera may show a faint reactivity only with the 12-kD band (Figure 2). Therefore, the assay should be interpreted as positive only when more than two bands are detected. Finally, care must be taken to interpret as positive the 12-, 16-, 18-, 21-, and 28-kD bands because there may be reactivity with lower molecular weight bands in less than 1% of the patients (Villota GE and others, unpublished data). A similar finding has also been reported for the EITB assay. The detection of additional bands with some sera is consistent with the lower specificity of the semi-crude T. solium preparations in ELISAs in which discrimination between the reactivity of specific and non-specific antigens is not possible.

Some of the patients with suspected NCC may have had a previous infection with T. solium metacestodes. Most (78%) of them had calcified lesions, a common feature in this group of patients in which a precise diagnosis is usually problematic. However, from a clinical perspective these patients are managed symptomatically (i.e., anticonvulsants, shunts, steroids) because the absence of detectable antibodies against T. solium metacestodes usually indicates that there is no need for anti-helminthic medications.

In summary, VF was evaluated by Western blotting under conditions that provided a sensitivity and specificity similar to that of a commonly used EITB assay. In Colombia, the commercial EITB assay is at least three times more expensive than the Western blot assays used in this study. Given the relative ease of obtaining VF from cyst-infected swine in developing countries where NCC is endemic, this assay may be an alternative method for the diagnosis of NCC until recombinant antigens that have the same sensitivity and specificity are available at affordable costs.

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