Multiantigen Print Immunoassay for Comparison of Diagnostic Antigens for *Taenia solium* Cysticercosis and Taeniasis
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One of the best-characterized tests for the diagnosis of neurocysticercosis is the enzyme-linked immunoelectrotransfer blot assay, developed at the CDC, which uses lentil lectin-purified glycoproteins (LLGPs) extracted from *Taenia solium* cysticerci. The purification of the LLGP antigens has been difficult to standardize, and the polycrylamide gel system used for the immunoblot assay is not easily transferable to other laboratories. In this study, we developed a multiantigen printing immunoassay (MAPIA) to compare the performance of multiple recombinant *Taenia solium* proteins with the potential for the detection of cysticercosis and taeniasis. We prepared MAPIA strips using six cysticercosis and two taeniasis diagnostic proteins and compared the performance of the proteins with sera collected from defined cysticercosis and taeniasis cases. Of the six cysticercosis antigens, rT24H performed well in detecting cases with two or more viable cysts in the brain (sensitivity and specificity, 97% and 99.4%, respectively); the use of a combination of cysticercosis antigens did not improve the sensitivity of the test and decreased the specificity. None of the antigens could differentiate the different clinical presentations of cysticercosis. Both of the taeniasis antigens (rES33 and rES38) had the same sensitivity of 99.4% and specificities of 93.9% and 94.5%, respectively. Some cross-reactivity against rES33 and rES38 was found, especially with sera from cases infected with *Schistosoma mansoni*. We conclude that MAPIA is a simple and effective tool that may be used to compare antibody responses to different cysticercosis and taeniasis antigens and, in this case, may be useful for the rapid detection of *T. solium* cases.

Excellent laboratory methods with high specificities and sensitivities for the immunodiagnosis of neurocysticercosis and taeniasis exist. The enzyme immunoelectrotransfer blot (EITB) for cysticercosis is accepted as the “gold standard” assay for the serological identification of cysticercosis (16, 19). Unfortunately, the test employs complex native proteins in immunoblot assay formats, and therefore, the tests are not easily adaptable to field use. Over the last 10 years we systematically purified and cloned the diagnostic glycoproteins expressed in the lentil lectin glycoprotein fraction. We found that the seven diagnostic proteins are members of three antigenic protein families: the GP50, GP24, and 8-kDa families. The recombinant proteins or synthetic peptides identified in the first-generation assays are available for further comparative analysis.

Many of these recombinant proteins (rGP50 and rT24H, used for the diagnosis of cysticercosis, and rES38 and rES33, used for the diagnosis of taeniasis) and synthetic peptides (sTSRS1, sTS18var1, sTSRS2var1, and sTS14, used for the diagnosis of cysticercosis) have been evaluated by EITB or enzyme-linked immunosorbent assay (ELISA) and have performed well (3, 7–9, 11, 18). Unfortunately, the development of diagnostic methods that use all of these proteins will be expensive and may be unnecessary. Nonetheless, an assay that uses more than one diagnostic protein may be required to maximize the sensitivity or to investigate associations that may exist between immunoreactivity and clinical signs, symptoms, and status. A method for the simultaneous, side-by-side comparison of these recombinant proteins and synthetic peptides is needed. Unfortunately, the classical assay formats, ELISA and EITB, are not adequate for antigen comparison studies. Several of the *Taenia solium* recombinant proteins (e.g., rGP50, rES33, and rES38) or synthetic peptides (sTSRS1, sTS18var1, sTSRS2var1, and sTS14) comigrate in the EITB. An ELISA format that combines more than one protein would not be useful because the responses to individual proteins cannot be dissected.

The multiantigen printing immunoassay (MAPIA) or line immunoassay is an antibody detection method that employs the direct application of proteins sprayed onto nitrocellulose membranes in lines, followed by the performance of classical antibody detection methods, typically by using an enzyme-conjugated anti-immunoglobulin and precipitating enzyme substrate. MAPIA permits the detection of antibodies to many unrelated antigens in a single assay (13, 15, 17). In this study,
we used a MAPIA to compare the performance of different recombinant protein and synthetic peptide antigens for the serological detection of cysticercosis and taeniasis.

MATERIALS AND METHODS

Chemicals and reagents. All reagents were reagent grade or better and unless otherwise noted were obtained from Mallinckrodt (St. Louis, MO). Tris was obtained from MP BioMedicals (Solon, OH). The horseradish peroxidase (EC 1.11.17)-conjugated goat anti-human IgG conjugate was prepared in our laboratory, as described previously (20, 21).

_Taenia solium_ antigens. Recombinant proteins rGP50, rES33, and rES38 were expressed in S21/S97 cells by using a baculovirus system. Similarly, the extracellular domain of T24, rT24H, was expressed in _Tni_ cells (8, 9, 11). Synthetic peptides sTsRS1, sTs18var1, and sTs14 were chemically synthesized (AnaSpec, San Jose, CA) (3, 7, 18). sTs18var1 was solubilized in 50 mM dithiothreitol–0.05 M HEPES–0.1 M NaCl to prevent polymerization via disulﬁde bonding (18). All of the cysticercosis protein antigens (rGP50, rT24H, sTsRS1, sTs18var1, and sTs14) were treated with a sodium dodecyl sulfate (SDS; Bio-Rad, Hercules, CA) solution in 1:2 protein mass ratios. The treated mixtures were heated at 65°C for 15 min and then were desalted into phosphate-buffered saline (PBS) with 2-ml Zeba desalt spin columns (Thermo Scientific, Rockford, IL). rES33 and rES38 were dissolved in PBS and in 20 mM Tris-HCl, pH 8.0–0.2 M NaCl, respectively, without SDS treatment.

_Sera_. A total of 274 serum samples were collected at the Instituto de Ciencias Neurologicas, Lima, Peru, from patients presenting with clinical symptoms of neurocysticercosis. The diagnosis of cysticercosis was confirmed by imaging of the brain by computed tomography (CT) or magnetic resonance imaging (MRI) (14). The four 8-kDa antigens showed similar reactivities with the cysticercosis-positive serum pool. The optimum concentration of each antigen was shown.

RESULTS

The optimal concentration of each protein antigen was determined separately by visual examination of the signal versus the noise by using a positive serum sample pool and two negative serum samples (one sample was from a patient with echinococcosis and the other was a negative serum sample pool). The optimum concentrations of antigens were determined to be as follows: rGP50, 0.1 ng/mm; rT24H, 2.5 ng/mm; sTsRS1, 2 ng/mm; sTs18var1, 0.45 ng/mm; sTsRS2var1, 0.5 ng/mm; sTs14, 3 ng/mm; rES38, 1.25 ng/mm; and rES33, 2.5 ng/mm (Fig. 1).

Reading of the MAPIA strips was straightforward, and the level of agreement between the two readers was high. The kappa values for rGP50, rT24H, sTsRS1, sTs18var1, sTsRS2var1, sTs14, rES38, and rES33 were 0.99, 0.96, 0.96, 0.91, 0.98, 0.92, and 0.98, respectively.

We evaluated the sensitivity of the individual antigens using sera from patients with clinically confirmed cases of cysticercosis. The rT24H antigen had the highest reactivity for all presentations of cysticercosis (Table 1). The four 8-kDa antigens showed similar reactivities with the cysticercosis-positive sera. They were also the least recognized proteins among the larval-stage proteins by sera from all different categories of patients with neurocysticercosis. The positivity of the sera was the highest with sera from cases with two or more viable cysts and was the lowest with sera from cases with only calcified cysts. In sera from cases with two or more viable cysts, the use...
of more than one antigen did not improve the sensitivity of detection. Overall, the use of rT24H alone was sufficient. However, for the detection of cases with a single viable cyst, any combination of antigens resulted in a higher sensitivity compared to that achieved with the use of rT24H alone (sensitivities, 67.7% and 60%, respectively), and the increase in sensitivity was contributed by a combination of all four 8-kDa antigens. No specific reactivity patterns could be correlated to the different clinical presentations of neurocysticercosis (e.g., the presence of calcified or degenerating cysts).

We evaluated the specificities of the individual antigens using a serum panel that consisted of sera from healthy individuals and sera from cases diagnosed with parasitic diseases other than cysticercosis and taeniasis. rGP50 had the greatest specificity; no sera from the specificity panel reacted with the antigen (Table 2).

To evaluate the ability of the recombinant adult worm antigens to detect taeniasis, we evaluated sera from cases with confirmed taeniasis and looked for reactivity with two tapeworm-specific proteins, rES38 and rES33. Of 162 specimens tested, 1 serum specimen did not react to either rES38 or rES33 (sensitivity of the taeniasis MAPIA, 99.4%). The specificity was evaluated by using the specificity panel; and the specificities of rES33 and rES38 were 93.9% and 94.5%, respectively; 17 serum samples from cases with schistosomiasis reacted with rES38; 19 serum samples reacted with rES33. Sera from six cases of confirmed Taenia saginata infection did not react with rES38 or rES33 (Table 2). In this matter, use of a combination of Taenia antigens did not improve the sensitivity or the specificity. Although the sera from patients with cysticercosis and taeniasis were collected on the basis of the findings of cysts by a CT scan or MRI and the recovery of adult T. solium worms, respectively, these defined sera were not tested at the same time for both infections. By using the sera from cases with cysticercosis and two or more viable cysts, 81% of the subjects had antibody against rES33 and 83% had antibody against rES38. In subjects with active taeniasis, the reactivities to rGP50, rT24H, sTsRS1, sTs18var1, sTsRS2var1, and sTs14 were 88.9%, 93.2%, 35.2%, 34%, 42%, and 29.6%, respectively. All of these 53 cases, including 25 cases without neurological symptoms and negative neuroimaging findings, were seropositive for cysticercosis and taeniasis antigens.

## Discussion

In this study, we demonstrated that a MAPIA with eight different recombinant proteins and synthetic peptides derived from both the larval and the adult worm stages of T. solium can be used to compare the relative diagnostic potentials of the different protein antigens. We found that the MAPIA with rT24H had performance characteristics that were comparable to those of the LLGP EITB, the accepted gold standard method for the serological detection of cysticercosis (5, 19). The overall performance of this MAPIA was also comparable to that of the other assays that we developed, such as the 8-kDa EITB (18), the rES38-rES33 EITB (11, 12), and the rGP50 and sTs18var1 FAST (Falcon assay screening test) ELISA (3).

The MAPIA for cysticercosis and taeniasis presented here was easily adapted from the original descriptions of MAPIA methods (13, 15, 17). Pretreatment of all of the cysticercosis protein antigens (rGP50, rT24H, sTSRS1, sTS18var1, sTSRS2var1, and sTS14) with SDS was required prior to printing to linearize the epitopes and to decrease the nonspecific binding. SDS treatment of the taeniasis proteins (rES33 and rES38) was not necessary and actually reduced the reactivities to the proteins.

### Table 1. Sensitivity of MAPIA with cyst-stage proteins and peptides for detecting neurocysticercosis

<table>
<thead>
<tr>
<th>Cysticercosis classification</th>
<th>sTs14</th>
<th>sTsRS2</th>
<th>sTs18var1</th>
<th>sTsRS1</th>
<th>rT24H</th>
<th>rGP50</th>
<th>Any 8-kDa antigen</th>
<th>Any 8-kDa antigen or rT24H</th>
<th>Any 8-kDa antigen or rGP50</th>
<th>rT24H or rGP50</th>
<th>Any antigen</th>
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</thead>
<tbody>
<tr>
<td>Two or more viable cysts (n = 100)</td>
<td>80</td>
<td>77</td>
<td>87</td>
<td>81</td>
<td>97</td>
<td>93</td>
<td>92</td>
<td>97</td>
<td>94</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>Single viable cyst (n = 15)</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>47</td>
<td>60</td>
<td>53</td>
<td>53</td>
<td>67</td>
<td>67</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Calcified cysts (n = 95)</td>
<td>33</td>
<td>34</td>
<td>34</td>
<td>27</td>
<td>73</td>
<td>44</td>
<td>46</td>
<td>80</td>
<td>52</td>
<td>74</td>
<td>81</td>
</tr>
<tr>
<td>Degenerating cysts (n = 64)</td>
<td>33</td>
<td>31</td>
<td>39</td>
<td>33</td>
<td>72</td>
<td>32</td>
<td>48</td>
<td>72</td>
<td>59</td>
<td>73</td>
<td>77</td>
</tr>
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</table>

### Table 2. Specificity of MAPIA with cyst-stage proteins and peptides

<table>
<thead>
<tr>
<th>Serum panel</th>
<th>sTs14</th>
<th>sTsRS2</th>
<th>sTs18var1</th>
<th>sTsRS1</th>
<th>rT24H</th>
<th>rGP50</th>
<th>Any 8-kDa antigen</th>
<th>Any 8-kDa antigen or rT24H</th>
<th>Any 8-kDa antigen or rGP50</th>
<th>rT24H or rGP50</th>
<th>Any antigen</th>
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<tr>
<td>Normal sera</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>U.S. residents (n = 100)</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>6</td>
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<td>Egyptian residents (n = 15)</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Heterologous infection sera</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>S. mansoni (n = 101)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>5</td>
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<tr>
<td>Others (n = 37)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Specificity (%; n = 311)</td>
<td>99.7</td>
<td>99.4</td>
<td>98.7</td>
<td>99.4</td>
<td>100</td>
<td>97</td>
<td>96</td>
<td>97</td>
<td>99.4</td>
<td>96</td>
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</table>
During the development stage of the project, we recognized one major limitation in the antigen printing process. The optimum range of concentrations of the antigens was narrow and the final concentration that we used was very low; therefore, small pipetting errors were magnified and resulted in a lack of specificity. It was necessary to prepare a small batch of strips to ensure the sensitivity and the specificity of each lot before a large number were printed. Because of this limitation, we believe that MAPIA may not be useful as an assay platform per se, and its use may best be limited to antigen comparison studies.

MAPIA is uniquely suited to studies that require the evaluation of responses to multiple antigens from more than one organism or stage. Here, we measured the serological responses to both the larval and the adult stages of T. solium. The rT24H antigen performed the best for the detection of human cysticercosis; no antigen performed better than another for the detection of the different clinical states of cysticercosis. Also, the reading of the rT24H MAPIA results had the highest level of agreement between the two readers. On the basis of these observations, the rT24H antigen is the candidate of choice for use in the MAPIA for the detection of cysticercosis in future studies. rGP50 performed similarly in the MAPIA, but a few anecdotal reports of false-positive reactivity to native GP50 have been reported, thereby reducing the acceptance of GP50 (1, 10).

In this study, for the first time, we observed that people with cysticercosis have antibodies against taeniasis antigens and vice versa. Eighty-one to 83% of the subjects with two or more viable cysts had detectable antibodies against rES33 and rES38. Conversely, all subjects who had taeniasis and for whom detailed information of their neurocysticercosis status was available had strong antibody reactions to rGP50 and rT24H. Twenty-five of these 53 subjects had no history of neurocysticercosis, suggesting that these antibodies may represent a transient antibody response (6). The implications of these findings are not clear, and we do not know the longevity of antibodies against rES33 or rES38 or if most persons with taeniasis will also have cysticercosis.

In conclusion, we report on the development and evaluation of a MAPIA that permits the simultaneous comparison of several antigens for the serological detection of both cysticercosis and taeniasis. This method can be used in epidemiological studies to map cases and determine the seroprevalence of both cysticercosis and taeniasis. We also showed that rT24H can be used alone for the detection of cysticercosis. Given the ease of preparation and performance, we anticipate the production of a method that uses rT24H+rES38/ES33 for the detection of T. solium infections.

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