DEVELOPMENT OF A SEROLOGIC ASSAY TO DETECT TAENIA SOLIUM TAENIASIS

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Abstract. We developed a serologic assay to identify adult Taenia solium tapeworm carriers using excretory/secretory (TSES) antigens collected from in vitro cultured T. solium tapeworms. To identify taeniasis-specific antigens we used an immunoblot assay with serum samples from T. solium tapeworm carriers and cysticercosis patients. Antigens were identified that reacted with antibodies present in serum samples from taeniasis cases and not with those from cysticercosis patients. Using serum samples collected from persons with confirmed T. solium tapeworm infections, the test was determined to be 95% (69 of 73) sensitive. Serum samples (n = 193) from persons with other parasitic infections, including T. saginata tapeworm infections, do not contain cross-reacting antibodies to TSES, indicating that the assay is 100% specific. These data suggest that the immunoblot assay using TSES antigens can be used to identify persons with current or recent T. solium tapeworm infections and provides a new, important tool for epidemiologic purposes, including control and prevention strategies.

Humans can be infected with both the adult worm and larval forms of the cestode Taenia solium, causing taeniasis and cysticercosis, respectively. Humans, who are the natural definitive host for the adult tapeworm, develop taeniasis after eating raw or undercooked pork infested with the larval stages of the parasite. Both pigs and humans can develop cysticercosis by ingesting eggs passed in the feces of a tapeworm carrier. Consequently, cysticercosis can be acquired in any variety of cultural and socioeconomic conditions where there is close contact with a taeniasis carrier. Identification and treatment of taeniasis infections can be difficult to detect since they are usually asymptomatic. Therefore, accurate and sensitive detection of taeniasis cases becomes a critical element for developing successful control strategies for cysticercosis.1,2

Classically, taeniasis has been detected by direct parasitologic examination of stool samples. Detection methods, based on microscopic observation of eggs or proglottids in feces, are neither sensitive nor specific.3,4 Direct examination of Taenia eggs is equivocal and requires examination of expelled proglottids for speciation.5 Recently, coproantigen detection assays have been developed and were found to greatly increase the sensitivity of detecting taeniasis cases.6,7 The coproantigen assay is an antigen-capture ELISA6,8 that uses hyperimmune rabbit sera produced against somatic extracts of tapeworms to capture and detect parasite antigens excreted in stool samples. This assay, which has been used successfully in field situations,9 was shown to be 99% sensitive and greater than 99% specific for Taenia sp. One of the few disadvantages of this technique is that it is unable to distinguish T. solium and T. saginata infections.6 More recently, a method using DNA probes specific for T. solium or T. saginata was developed that uses species-specific primers to differentiate these two tapeworm infections.10,11 This technique relies on the amplification of parasite DNA obtained from parasite eggs or proglottids present in the stool sample. Although the polymerase chain reaction can detect the presence of a single egg,11 the intermittent passage of eggs in the stool limits the usefulness of this assay.

Independent of the detection method used, collection and examination of stool samples for identification of taeniasis cases is associated with several problems. Primarily, collection of fecal samples carries with it the potential for exposure to and infection with Taenia eggs present in the sample. In addition, collection of stool samples is often difficult and culturally unacceptable in many places where epidemiologic studies on cysticercosis and taeniasis are conducted.

Considering the problems associated with stool collection and manipulation, we investigated the possibility of developing a serologic test for detection of T. solium taeniasis. Early work on human intestinal taeniasis indicated the presence of antibodies to T. saginata.12 However, a number of factors, including sensitivity and specificity of the antigens and methods available, limited their application in diagnosis. More recently, work on canine taeniasis has indicated the presence of and diagnostic potential for antibodies to a variety of antigens, including tapeworm excretory secretory (ES) products.13,14 No previous work has reported serodiagnosis of human taeniasis caused by T. solium. In this study, we attempted to develop a serologic assay using ES products from T. solium tapeworms (TSES) that would detect tapeworm carriers in a sensitive and specific manner.

MATERIALS AND METHODS

Reagents and chemicals. Minimum essential media (MEM) used for culture of tapeworms was obtained from GIBCO (Grand Island, NY). Protease inhibitors pepstatin and leupeptin were obtained from Calbiochem (La Jolla, CA) and Pefabloc® was obtained from Boehringer Mannheim (Indianapolis, IN). All reagents for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were reagent grade or better, and unless otherwise noted were obtained from Mallinckrodt (Orangeburg, NJ). The horseradish peroxidase (EC 1.11.17)–conjugated goat anti-human IgG conjugate was prepared in our laboratory as described.15

Parasite materials. Culture of T. solium tapeworms. Taenia solium tapeworms were harvested from immunosuppressed hamsters as described.16 Briefly, adult worms, 20–25 cm in length, were harvested from immunosuppressed hamsters

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approximately 6–8 weeks after infection with porcine *T. solium* cysts. Tapeworms were washed in antibiotics (penicillin and streptomycin) and placed into 25-cm² tissue culture flasks in MEM supplemented with penicillin/streptomycin and fungizone. Culture supernatants containing TSES antigens were collected following *in vitro* culture of *T. solium* tapeworms. For most experiments, TSES was collected after the first 24 hr of culture. This material was concentrated approximately 10-fold using a PM-10 membrane (Amicon, Beverly, MA) and stored frozen. After thawing, protease inhibitors were added. The ES preparations used in some early experiments represented pooled supernatants collected from days 5 to 16 of culture. The media was changed every 8 hr for the first 24 hr, then every 24 hr for the next two days. After the first 72 hr, the culture media was removed and replaced at five-day intervals. The culture supernatants from days 5 to 16 were pooled, concentrated with polyethylene glycol (molecular weight = 6,000), and lyophilized. The resultant material containing TSES antigens was frozen at −70°C, then reconstituted with 5.0 ml of distilled water containing final concentrations of the following protease inhibitors: leupeptin (1.0 µg/ml), pepstatin (1.0 µg/ml), and Pefabloc® (1 mM). Other preparations of ES were evaluated that represented supernatants collected after differing periods of culture, ranging from three to 15 days.

Approval was granted for the humane use of animals at the institutions where animal research was conducted (Salford University and Universidad Peruana Cayetano Heredia).

*Taenia solium* cyst extracts were prepared as described. Lentil lectin unbound and bound fractions were used in immunoblot experiments and loaded onto gels at 0.2 and 0.05 µg/mm, respectively.

**Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting.** The SDS-PAGE and immunoblot procedures were performed as described. Briefly, TSES antigens were treated for electrophoresis with 1% SDS, 0.1% bromophenol blue in 0.01 M Tris-HCl, pH 8.0, at 65°C for 15–20 min and loaded at 4 µl/mm. For analysis of individual serum samples, blots were cut into identical 3-mm strips. Cut strips were stored in the presence of 0.1% NaN₃ at −70°C until used. Sera were diluted 1:100 in phosphate-buffered saline/5% non-fat dry milk/0.3% Tween and incubated with the blotted antigens for 1 hr at room temperature. In isotype experiments, TSES-specific isotypes were detected using mouse monoclonal antibodies directed against the individual human isotypes, as described.

**Serum samples.** Serum samples from only persons known to be infected with *T. solium* tapeworms were used to develop and evaluate the sensitivity of the test. Serum samples were collected from individuals infected with *T. solium* tapeworms in Guatemala (n = 44), Peru (n = 26), and Indonesia (n = 3). In some experiments, a taeniasis-positive pool was used, which was made from five serum samples from persons with confirmed *T. solium* taeniasis. A cysticercosis positive pool was prepared from six persons with computed tomography–confirmed cysticercosis. Individual sera from persons with cisticercosis were collected in Bolivia (n = 23).

In immunoblot experiments examining the stage specificity of the TSES antigens, a serum sample was used that was obtained from a patient who was confirmed to have taeniasis, but was serologically negative for cisticercosis using the cysticercosis immunoblot. This particular serum sample was used because antibodies in this sample reacted only with the target diagnostic TSES antigens in the ES preparation. All individual disease-specific serum samples were selected on the basis of parasitologic confirmation of infection. All efforts were made to include individual serum samples associated only with one disease. Particularly, in selecting heterologous infection serum samples, we tried to include only samples from *T. solium*-free geographic regions; however, this was not possible in all cases. The *T. saginata* infection sera were collected from individuals in Poland (n = 6) and in Peru (n = 3). In Peru, parasitologic diagnosis of *T. saginata* infection was confirmed by direct examination of proglottids. Of the 69 echinococcosis sera used, 59 were from individuals infected with *E. granulosus* and 10 from individuals infected with *E. multilocularis*. A pool of normal human sera was prepared from 50 healthy U.S. residents who had no travel history to *Taenia*-endemic areas.

All serum samples were collected after obtaining informed consent and approval from the Internal Review Boards at the Centers for Disease Control and Prevention, Salford University, University of San Carlos, Guatemala, Universidad Peruana Cayetano Heredia, and The Johns Hopkins University.

### RESULTS

In the initial experiments, we investigated the validity of using TSES antigens in a serologic assay to detect taeniasis cases. To identify antigens that are specific for taeniasis and not cisticercosis, ES antigens were first separated by SDS-PAGE and evaluated by immunoblot using pools of serum prepared from persons infected with taeniasis or cisticercosis (Figure 1). Antigens that reacted with taeniasis antibodies, but not cisticercosis antibodies, were identified as potential diagnostic targets. A group of two antigens with *M* values of 32,700 and 37,800 were identified as potential diagnostic antigens and appeared to be specific for taeniasis.

We chose to examine TSES initially because of the reduced antigenic complexity present in these samples compared with that of extracts prepared from intact tapeworms. The TSES collected following the first 24 hr of culture was the least complex preparation that we examined. Evaluation of culture supernatants collected following several days (3–16) of culture showed that these preparations were much more complex. Interestingly, the 32.7-kD and 37.8-kD antigens were present in all preparations of TSES that we examined, suggesting that these antigens are synthesized continuously by the tapeworm. An additional antigen with an *M* of 42,100 was present in one TSES preparation collected from days 5 to 16 of culture.

Individual serum samples from taeniasis and cisticercosis patients were evaluated for reactivity with the TSES diagnostic antigens (Figure 2A and Table 1). Ninety five percent (69 of 73) of samples tested from parasitologically confirmed *T. solium* tapeworm infections contained antibodies to the TSES antigens. Typically, if a sample was determined to be positive, both the 32.7-kD and the 37.8-kD antigen were recognized. In TSES preparations that also contained the larger 42.1-kD antigen, all three antigens were recog-
nized by antibodies present in positive samples (Figure 2A), suggesting that these three antigens may be related to each other. Conversely, evaluation using serum samples from patients with cysticercosis showed that very few of these samples contain antibodies that reacted with the target ES antigens (Figure 2D). One of 23 samples tested in these experiments contained antibodies that reacted with the TSES antigens. Some cysticercosis patients may also be tapeworm carriers; therefore, it was not unexpected to detect antibodies to T. solium ES antigens in some samples.

We investigated the specificity of the TSES immunoblot assay first with regard to differentiation of T. solium and T. saginata infections. To determine if T. saginata tapeworm carriers generate antibodies to TSES antigens, particularly the target diagnostic antigens, serum samples from confirmed T. saginata tapeworm carriers were examined for reactivity with TSES antigens (Figure 2B and C and Table 1). For these experiments, serum samples from T. saginata tapeworm-infected persons were collected in areas where T. solium is not present (Poland, n = 6) (Figure 2B) and in areas where T. solium is endemic (Peru, n = 3). There were no cross-reacting antibodies present in any T. saginata samples that recognized any TSES antigens, including the TSES diagnostic antigens.

Serum samples from patients with other parasitic diseases were also examined for antibodies to the TSES antigens (Table 1). None of the 193 samples examined contained antibodies that reacted with the target ES antigens. The serum battery included 69 serum samples from patients with echinococcosis, and seven serum samples from patients infected with Hymenolepis nana. Some serum samples from echinococcosis patients contained antibodies that reacted with other higher molecular weight antigens in the ES mixture, but not with the diagnostic antigens.

Results from all of the serum samples examined are shown in Table 1. We used these data to calculate a measure of assay performance: the positive predictive and negative predictive values.\(^{20}\) The calculated positive predictive value was 100% (69 of 69) and the negative predictive value was 98% (193 of 197). The cysticercosis-positive samples from Bolivia were excluded from these calculations since they can not be classified as either true positives or true negatives.

Completion of the T. solium life cycle requires maturation of two different parasite stages through two different hosts. Therefore, we wanted to determine if the diagnostic adult-stage ES antigens were also expressed during the cyst stage. The lentil lectin unbound and bound fractions from cyst extracts were examined for the presence of the analogous TSES antigens using a taeniasis-specific serum sample (Figure 3). The taeniasis-specific antibodies did not react with

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**Figure 1.** Secretion or excretion of proteins by *Taenia solium* tapeworms that are immunogenic only in taeniasis infections but not in cysticercosis infections. The *T. solium* ES proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and either stained with silver stain (lane 1) or transferred to nitrocellulose and then blotted with infection-specific serum samples: *T. solium* taeniasis samples (lane 2), or cysticercosis samples (lane 3). kDa = kilodaltons.

**Figure 2.** Specific detection of taeniasis carriers by an immunoblot assay using excretory secretory products from *Taenia solium* tapeworms (TSES). Immunoblot experiments using TSES were performed to assess responses in individuals with either taeniasis caused by *T. solium* (A) or *T. saginata* (B and C), or cysticercosis (D). *Taenia saginata* serum samples were collected in Poland (B), an area that is not endemic for *T. solium*, or in Peru (C), an area that is also endemic for *T. solium*. Locations of the target diagnostic bands are marked with brackets. Positive samples are marked with a star (in D).
In this study we demonstrate that an immunoblot assay using TSES can detect *T. solium* tapeworm carriers in a highly sensitive and specific manner. The sensitivity of the assay was determined to be 95% (69 positive/73 infected) using serum samples from patients with *T. solium* tapeworm infections. Not only is the immunoblot assay sensitive, but it is 100% specific (n = 193). The test does not falsely identify persons with other cestode infections, such as *T. saginata*, *Echinococcus*, or *H. nana* infections. An additional merit of the assay is that it is serologic, thus eliminating many social and cultural problems associated with collection of other samples. Preliminary results from an ongoing study in Peru indicate that serum collected by fingerstick will substitute adequately for a venous blood sample, further simplifying sample collection.

Our data suggests that presence of antibodies to TSES antigens represents a current or recent infection with a *T. solium* tapeworm, even when antibodies are present in persons with cysticercosis. Several lines of evidence support our contentions. Most serum samples from persons with cysticercosis patients do not contain antibodies that react with the ES antigens. The diagnostically important TSES antigens appear to be synthesized only during the adult stage of the parasite and not by the larval stage of the parasite. Epidemiologic data suggest that autoinfection with shed eggs from taeniasis carriers resulting in coinfection with the larval stage is plausible. In this study, we did not attempt to examine the degree of coinfection between larval and adult forms of the parasite. A more precise determination of the rates of coinfection can be better determined later in another study.

Any assay to detect *T. solium* tapeworm carriers needs to be a rapid assay in a format capable of analyzing numerous samples in a short period of time. Further purification of the diagnostic TSES antigens, perhaps resulting in production of recombinant antigens, is needed to develop a faster, field adaptation of this assay. Our current efforts are focusing on purification of the specific diagnostic antigens from TSES preparations to fashion the immunoblot into a simpler assay. Future evaluation of this assay in disease-endemic areas will provide a more exacting validation of the test. Studies are under way to determine the rates of both false-positive and false-negative results using this test. False-positive results may exist because this assay is a serologic test, and antibodies to parasitic infections can remain after elimination of parasites. Future studies will address the persistence of

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**Table 1**

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<th>Infection*</th>
<th>No. of samples tested</th>
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*Filariasis sera were collected from individuals infected with onchocerciasis (n = 26) and lymphatic filariasis (n = 4, caused by *Wuchereria bancrofti*). Schistosomiasis infection sera were collected from persons with *S. mansoni* (21), *S. haematobium* (8), and *S. japonicum* (8) infections.

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**Figure 3.** Immunoblot assay showing that target diagnostic proteins (secretory excretory products from *Taenia solium* tapeworms [TSES]) are present only in fractions derived from tapeworm extracts and not present in cyst extracts. TSES (lanes 1), cyst-derived, lentil lectin unbound (lane 2), or lectin bound (lane 3) extracts were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose, then probed with taeniasis- or cysticercosis-specific serum. kDa = kilodaltons.
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Figure 4. Immunoblot assay showing that IgG1 and IgA are the predominant isotypes produced against the excretory secretory products of *Taenia solium* tapeworms (TSES diagnostic proteins). Specific human immunoglobulin isotype responses to TSES were assessed by immunoblot using three individual taeniasis-infection serum samples and detected with monoclonal mouse reagents specific for each human isotype. The immunoglobulin isotypes are labeled as follows: IgG1 = 1; IgG2 = 2; IgG3 = 3; IgG4 = 4; IgM = M; IgA = A; IgE = E. Locations of the target diagnostic bands are marked with brackets.

Antibodies after elimination of tapeworms. Currently, we have developed a diagnostic tool that can be used to identify persons with *T. solium* tapeworm infections. In conclusion, we believe this assay is a new, important tool that can be used in epidemiologic studies to address transmission dynamics of taeniasis and cysticercosis and to develop strategies to interrupt transmission of these diseases.

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REFERENCES


