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Antigens from *Taenia crassiceps* Cysticerci Used in Complement Fixation, Enzyme-Linked Immunosorbent Assay, and Western Blot (Immunoblot) for Diagnosis of Neurocysticercosis

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Antigens from *Taenia solium* cysticerci for the immunodiagnosis of cysticercosis are scarce and difficult to obtain. We studied the reliability of antigens from *Taenia crassiceps* cysticerci as a substitute for those from *T. solium* in three diagnostic tests: complement fixation, enzyme-linked immunosorbent assay, and Western blot (immunoblot). Precision rates of the three tests of 93, 99, and 96%, respectively, were found. Cysticerci from *T. crassiceps* can be easily obtained in large quantities and can be effectively used for the diagnosis of human neurocysticercosis.

Immunodiagnosis of neurocysticercosis (NCC) depends on the search for antibodies against cysticerci antigens in cerebrospinal fluid (CSF) from patients suspected of having the infection (2, 11, 13). Extracts from membranes of *Taenia solium* cysticerci provide the substratum for antibody detection. Although NCC is the most frequent parasitic disease of the nervous system, the only source for antigens of *T. solium* cysticerci for immunodiagnostic assays is derived from the parasites extracted from parasitized pork meat. The amount of cysticerci obtained from pork varies widely in relation to the parasite burden, and the extirpation of cysts from pork muscle is a laborious and time-consuming procedure. In addition to these technical difficulties, parasitized pork is difficult to obtain even in areas where the organism is endemic because farmers tend to hide sick animals to avoid confiscation; therefore, the existence of most infected pork is denied and the animals are sacrificed and sold clandestinely. Difficulties in obtaining cysticercal antigens have prevented the standardization of immunodiagnostic tests and the access of many patients to these diagnostic studies.

Antigens from *T. solium* cysticerci have a striking homology to those from *Taenia crassiceps* cysticerci (1, 8). In 1990 we reported the usefulness of antigens from *T. crassiceps* cysticerci as a substitute for antigens from *T. solium* cysticerci in the enzyme-linked immunosorbent assay (ELISA) for the diagnosis of human neurocysticercosis in CSF (10). Advantages tied to the use of antigens from *T. crassiceps* cysticerci as a substitute for *T. solium* cysticerci include the high indices of cross-reactivity of antibodies from patients with NCC with antigens from *T. crassiceps* cysticerci (9), the extreme rarity of *T. crassiceps* infections in humans (7), and the inexpensive and simple acquisition of large amounts of *T. crassiceps* cysticerci through the laboratory-adapted model of infection in mice (9, 14). Because of the fast asexual reproduction of *T. crassiceps* cysticerci by gemmation, within a few weeks thousands of cysts are obtained from a dozen parasites initially injected into the peritoneal cavity of a single mouse. In the study described here, we analyzed the accuracy of the three most frequently used immunodiagnostic tests for NCC, complement fixation (CF), ELISA, and Western blot (WB; immunoblot), in CSF using antigens from *T. crassiceps* cysticerci compared with the results obtained by the same tests using antigens from *T. solium* cysticerci.

One hundred CSF samples obtained by lumbar puncture from patients with NCC positive by CF, 100 CSF samples positive by ELISA, and 100 CSF samples positive by WB were selected from our CSF bank, where they had been kept frozen at −70°C for periods ranging from 1 to 18 months. In all cases the diagnosis of NCC had been confirmed by neuroimaging studies, either computed tomography or magnetic resonance, or both (2). One hundred CSF samples from patients with a comprehensive variety of neurological ailments in whom a diagnosis of NCC had been discarded by CSF analysis and neuroimaging studies were used as controls. Each test was performed as described previously (3, 4, 9) with antigens extracted from *T. solium* cysticerci. Afterward, the same studies were repeated with *T. crassiceps* cysticerci used as the source of antigens. The *T. crassiceps* cysticerci were extracted exactly as those of *T. solium* cysticerci, as described for each method.

The results obtained with antigens from *T. solium* cysticerci were taken as the standard values against which the results obtained with antigens from *T. crassiceps* cysticerci were compared for overall precision, prevalence, sensitivity, specificity, and predictive values of CF, ELISA, and WB for the immunodiagnosis of NCC. In the case of WB, CSF samples showing various reactive lines (from five to countless) with antigens within a molecular mass range of 45 to 110 kDa were taken as positive.

Of those 100 CSF samples from patients with NCC which gave positive results by the CF test with antigens from *T. solium* cysticerci, 86 continued to be positive when the test was repeated with antigens from *T. crassiceps* cysticerci. Of those 100 CSF samples from controls which gave negative results with antigens from *T. solium* cysticerci, all 100 continued to be negative with antigens from *T. crassiceps* cysticerci. Of those 100 CSF samples from patients with NCC which gave positive results by the ELISA with antigens from *T. solium* cysticerci, 97 continued to be positive when the test was repeated with antigens from *T. crassiceps* cysticerci. Of those 100 CSF samples from controls which gave negative results with antigens from *T. solium* cysticerci, all 100 continued to be negative with antigens from *T. crassiceps* cysticerci. Of those 100 CSF samples from patients with NCC which gave positive results by the WB test

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TABLE 1. Reliabilities of three immunodiagnostic assays for NCC in CSF with antigens from *T. crassiceps* cysticerci compared with results with antigens from *T. solium* cysticerci

Test	Overall precision (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Prevalence (%)
CF	93	86	100	100	88	50
ELISA	99	97	100	100	97	50
WB	96	93	98	98	93	50

with antigens from *T. solium* cysticerci, 93 continued to be positive when the test was repeated with antigens from *T. crassiceps* cysticerci. Of those 100 CSF samples from controls which gave negative results with antigens from *T. solium* cysticerci, 98 continued to be negative with antigens from *T. crassiceps* cysticerci, while 2 samples gave few reactive bands and were considered false positives.

The reliability of each test when antigens from *T. solium* cysticerci were replaced by antigens from *T. crassiceps* cysticerci is shown in Table 1.

In immunodiagnostic tests for NCC, the source of cysticercal antigens from the helminth *T. solium* can effectively be substituted by those from the helminth *T. crassiceps*. Overall precisions are 93% for CF, 99% for ELISA, and 96% for WB when compared with the results for known positive or negative CSF samples from patients with NCC and controls (Table 1). The practical advantages of this substitution for the routine use of these antigens are (i) there is a unlimited source of *T. crassiceps* cysticerci, whereas there are restraints in obtaining *T. solium* cysticerci, and (ii) the cost of antigens would be minimal because *T. crassiceps* cysticerci can grow indefinitely in laboratory mice, in contrast to the technical and economic difficulties in obtaining infected pork, the only nonhuman host for *T. solium* cysticerci. The use of cysticerci from *T. crassiceps* represents a possibility for the standardization and widespread use of antigen preparations, particularly in areas where economic restraints prevent the use of expensive or sophisticated reagents; this point is particularly important because cysticercosis particularly affects people in lower socioeconomic strata. Technically, *T. crassiceps* cysticerci also have some advantages; that is, the cysts obtained from the laboratory-adapted model in mice are anembryonic and represent a hydatiform degeneration of the parasite, with unlimited growth of the cystic membranes. Therefore, the laborious procedure of individual separation of scoleces from the cysts is not required for antigen preparation, and the antigen extraction procedure from *T. crassiceps* cysticerci is faster and easier than that from *T. solium* cysticerci.

The precision values for each immunodiagnostic test used in the present study for the whole spectrum of NCC have been reported previously (3, 4, 9, 13, 15). They depend on the location of the parasite within the nervous system, the number of lesions, and the degree of the immune response of the host (15). In our experience, immunodiagnostic tests are not reliable with serum specimens, particularly because of the very low degree of specificity in patients and healthy subjects from areas where the disease is endemic (12), whereas in CSF specimens these tests have high degrees of specificity and their sensitivities largely depend on the presence of an inflammatory reaction in the subarachnoid space (2, 5, 15, 16). In general, the ELISA is more reliable than either CF or WB, but all of these tests have some advantages and some limitations over the others, so that, similar to the case for other parasitic diseases (6), the combined use of two immunodiagnostic tests offers an increase in diagnostic accuracy over the use of a single test. From our experience with the more than 10,000 CSF specimens that we have tested, we consider that in the everyday situation of neurological consultation, the simultaneous use of CF and ELISA for the testing of CSF specimens provides optimal diagnostic accuracy. A change in the use of antigens from *T. solium* cysticerci to the use of those from *T. crassiceps* cysticerci for routine diagnosis offers various technical and economic advantages, whereas the slight diminution in the precision values obtained by using antigens from *T. crassiceps* cysticerci compared with those obtained by using antigens from *T. solium* cysticerci is minimal when weighted against the advantages.

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