Serological techniques for the diagnosis of cysticercosis

M. D. PAMMENTER, E. J. ROSSOUW

Summary
Three techniques — an indirect haemagglutination test, a fluorescent antibody test and enzyme-linked immunosorbent assay (ELISA) — have been established for the detection of cysticercosis antibodies in the serum or cerebrospinal fluid of patients with cysticercosis. Results obtained using these techniques have been compared to determine the most successful serodiagnostic method. In each test there was a marked difference between the detection of active and of calcified cysts. The results obtained using the three tests were remarkably similar, and none had a statistically significant advantage over the others with regard to the detection of cysticercosis antibodies. However, ELISA appears to have certain other advantages.

Cysticercosis, a disease caused by the cystic stage of the pork tapeworm (*Taenia solium*), has been described as the most common parasitic disease affecting the central nervous system in the world. Under normal circumstances the adult worm lives in the intestine of man and the cystic stage takes place in pigs, which become infected by consuming eggs excreted in human faeces. The eggs hatch and release oncospheres which migrate through the tissues, where they develop into cysts. Humans occasionally ingest the eggs and become the intermediate host. While cysts may be found in various tissues, they appear to have a predilection for the central nervous system. Despite reports showing a high prevalence of cysticercosis in Black population groups and indicating the important role it plays in mental illness, the disease has never been regarded as a serious problem by the South African medical profession. Recently cysticercosis has received some prominent publicity, and the advent of new drugs to combat the disease has brought it to the fore again.

The condition is now treatable early and accurate diagnosis may be crucial, but diagnosis has always been problematic. Clinical symptoms include nausea, headache with disturbed vision, seizures, hemiparesis, raised intracranial pressure and papilloedema, but none of these is specific for cysticercosis. The presence of cysts in subcutaneous nodules confirms the diagnosis, but this feature is reported to be relatively rare.

Diagnosis by computed tomography (CT) is becoming increasingly popular and, with the advent of improved equipment, increasingly accurate. However, its use is restricted and in certain cases positive identification of cysts is still impossible.

Serological tests for cysticercosis have long been used as a diagnostic aid, and because of the lack of specific clinical symptoms they play a major role in its detection. In an attempt to find the most efficient diagnostic technique we recently established an enzyme-linked immunosorbent assay (ELISA) and an indirect fluorescent antibody test (FAT), and have compared their efficiency with that of a modification of the indirect haemagglutination test (HAT) originally developed by Proctor et al. The results obtained using these three assay systems are reported in this article.

Material and methods

Antigen

Cysts were removed from infected pork and stored at -86°C until required. The FAT antigen consisted of 6 μm thick sections of 2 - 4 cysts cut by cryomicrotomy. The same antigenic preparation was used in the HAT and the ELISA. Cysts were homogenized in 0,02M tris, 1,0 mM ethylenediamine tetra-acetic acid (pH 7,4), using a ground-glass Potter-Elvehjem homogenizer, until the outer membranous material of the cysts was completely homogenized. The homogenate was centrifuged at 105 000 × g to yield a supernatant which was used as the antigen.

The HAT

Cells were prepared as described by Hoq and Das. Human O-negative or O-positive cells were fixed with 2% glutaraldehyde and tanned with a 1/40 000 tannic acid solution. Antigen was attached to the cells at a concentration previously determined by titration. The HAT was performed in a total of 50 μl using standard procedures. Cerebrospinal fluid (CSF) or serum (diluted 1/10) was consecutively double-diluted with 2% bovine serum albumin (BSA) and mixed with an equal volume of a 1% sensitized cell suspension.

The FAT

The FAT was performed as recommended by Kawamura. Sections of cysts were incubated at 37°C with CSF or a 1/10 dilution of serum in phosphate-buffered saline (0,066M sodium orthophosphate, 0,85% NaCl, pH 7,2). After washing the sections were incubated with a 1/30 dilution of fluorescein-conjugated anti-human IgG plus IgM (Hoechst OTKG 05), with 0,05% Evans blue as a counterstain.

The ELISA

Flat-bottomed PVC immunoassay plates (Flow Laboratories 77-173-05) were coated with 50 μl antigen solution at room temperature for 2 hours in a moist container. The antigen solution contained 10 μg protein per millilitre of 0,05M sodium carbonate buffer, pH 9,6. After coating, the plates were washed with TST (0,05M tris, pH 8,0; 0,75M NaCl; 0,05% Tween 20) and blocked with 2% BSA in a sodium carbonate buffer.

Research Institute for Diseases in a Tropical Environment of the South African Medical Research Council, Durban

M. D. PAMMENTER, PH.D., Senior Chief Research Officer

E. J. ROSSOUW, DIP. MED. TECH., Technical Officer
Blocking was accomplished after 1 hour, following which plates were washed, dried and stored at -86°C. Plates were stored for up to 3 months without any apparent loss of activity.

The assay was conducted as described by Conradie and Mbhele\(^5\) with the following modifications: serum samples were tested at four dilutions (1/50, 1/100, 1/200 and 1/400) made with 4% normal pig serum in TST (the use of normal pig serum is critical; without it a variable background colour develops). An alkaline phosphatase-conjugated anti-human IgG (Sigma A 3150) diluted 1/500 with 4% pig serum was used and colour was developed using 'Sigma 104' substrate tablets (Sigma 104-105) in 1,0M diethanolamine and 0,5 mM MgCl\(_2\), pH 9.8, after which the absorbance at 405 nm (OD 405) was recorded. Two positive and negative controls were included in each assay plate.

The OD 405 of the negative controls was regarded as background and subtracted from the OD 405 of the appropriate dilutions of the positive controls and unknown samples to give a 'true' OD 405 reading. The positive control was deemed to have 1 280 units per microlitre and a standard curve of units versus 'true' OD 405 was constructed for each plate. From this curve the number of units in unknown samples was calculated.

**Results**

Fig. 1 is a graphic representation of the results of the three tests performed on sera obtained from patients with cysticercosis (the diagnosis being confirmed by either biopsy or a CT scan). The control group comprised 79 patients suffering from amoebic liver abscess. A complete clinical examination was performed on each patient in this group and all were found to be free of symtomatic neurological disorders or palpable nodules. Although admittedly this could not exclude asymptomatic cerebral or muscular cysticercosis, only 1 of the 79 sera tested gave a positive reaction to more than one test, and to all intents and purposes this group could therefore be regarded as free of cysticercosis.

Fig. 1 shows that, as is the case with all tests of this type, sensitivity must be weighed against specificity. We have selected the following cut-off points for positivity: (i) ELISA — 80 units and above; (ii) HAT — a positive reaction at a dilution of 1/40 or more; and (iii) FAT — 1 unit of fluorescence at a 1/10 dilution of serum. Using these values we were able to calculate the following sensitivities for the tests: ELISA 45.5%; HAT 33.3%; and FAT 48.9% (Table I).

**TABLE I. DETECTION OF ANTICYST ANTIBODIES IN THE SERUM AND CSF OF PATIENTS WITH CYSTICERCOSIS**

<table>
<thead>
<tr>
<th>Tests</th>
<th>ELISA</th>
<th>HAT</th>
<th>FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>20/44</td>
<td>17/51</td>
<td>22/45</td>
</tr>
<tr>
<td>Calcified cysts only</td>
<td>3/18</td>
<td>5/24</td>
<td>3/24</td>
</tr>
<tr>
<td>Active cysticercosis</td>
<td>12/17</td>
<td>11/20</td>
<td>12/15</td>
</tr>
<tr>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>25/45</td>
<td>16/51</td>
<td>12/50</td>
</tr>
<tr>
<td>Calcified cysts only</td>
<td>8/23</td>
<td>4/24</td>
<td>3/24</td>
</tr>
<tr>
<td>Active cysticercosis</td>
<td>12/15</td>
<td>8/16</td>
<td>5/14</td>
</tr>
<tr>
<td>Combined CSF and serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>45/89</td>
<td>33/102</td>
<td>33/94</td>
</tr>
<tr>
<td>Calcified cysts only</td>
<td>11/41</td>
<td>21/75</td>
<td>4/43</td>
</tr>
<tr>
<td>Active cysticercosis</td>
<td>24/32</td>
<td>19/36</td>
<td>17/29</td>
</tr>
<tr>
<td>Serum (control group)</td>
<td>1/79</td>
<td>1/79</td>
<td>5/79</td>
</tr>
</tbody>
</table>

**Results**

Similar results were obtained with CSF (Table I), but the sensitivity of the FAT was considerably less than with serum. From the results presented in Table I it is clear that there was a close correlation between the effectiveness of tests. The apparent
low sensitivity of the tests (Table I) can be explained by the status of the disease. The patients diagnosed as having cysticercosis could be divided into two groups, those with active cysts and those with calcified lesions only. There was a high sensitivity for active cysticercosis, but calcified cysts were difficult to detect serologically (Fig. 1, Table I).

Sera from patients with other parasitic diseases were also tested; no significant difference from the control subjects was found in the rate of positive titres in sera from patients infected with Schistosoma species (10/49, FAT) or Hymenolepis (1/9, FAT). However, the ELISA gave positive results for 7 of a total of 10 sera from patients with hydatid disease.

**Discussion**

Several tests for cysticercosis, with sensitivities ranging from 25% to 100% and false-positive rates of 2 - 25%, have been developed. These results compare favourably with the results obtained during the present study. While the overall sensitivity of the ELISA (50%) appears lower than has been reported by Diwan et al. (60%) and Arambulo et al. (75%), its sensitivity in the detection of active cysticercosis is at least equal (75%). The ELISA system used during this study had the advantage over that used by Diwan et al. that only one test was necessary. Those workers used a control ELISA with porcine soluble proteins as antigen; this was necessary to eliminate false-positive results caused by the development of high levels of background colour which it was suggested were due to host-related proteins being carried over with the cyst-antigen preparation. We have overcome this problem by using 4% normal pig serum as the diluent in the test. This resulted in an almost 50% reduction in the OD 405 values and reduced background colour to low levels being carried over with the cyst-antigen preparation. We have overcome this problem by using 4% normal pig serum as the diluent in the test. This resulted in an almost 50% reduction in the OD 405 values and reduced background colour to low levels. This resulted in an almost 50% reduction in the OD 405 values and reduced background colour to low levels (0 - 50 units), obviating the use of a control antigen. However, this procedure may decrease the potential sensitivity of the test.

It seems unlikely that the vast differences in test sensitivities reported by various workers are due solely to differences in laboratory technique. The selection of patients for assessment of the tests plays an important role. The results of this study clearly indicate that patients with calcified cysts only have low antibody levels and that inclusion of their sera lowers the calculated sensitivity. With modern equipment, calcified cysts are readily detected and many cases of cysticercosis which would previously have remained undiagnosed are now being detected. Because of the low antibody titres in these patients, serological tests appear to be less effective than has previously been reported.

Natural calcification of cysts is regarded as denoting spontaneous regression of the disease with return to normal and disappearance of symptoms. Subjects with calcified cysts would not be considered for treatment, and for this reason the inability of serological tests to detect calcified cysts in no way detracts from their usefulness.

The value of serological tests is not uniformly accepted, and the clinician must therefore know the implications of a laboratory result. In our tests a positive result was highly indicative of active cysticercosis requiring treatment. With the ELISA 75% of cases of active cysticercosis can be detected; this can be increased to 88% if all three tests are used. In about 13% of active cases a positive result was obtained in only one of the three tests, and these are the cases causing concern. If the test in question is the ELISA or the HAT the result is still highly suggestive of the presence of the disease, since false-positive results are rare in both these tests. However, the FAT would seem to have the highest false-positive rate (5 out of 79 results in the control group and 10 out of 49 in patients with schistosomiasis), so that a single positive FAT result may be regarded with some suspicion. Unfortunately we were unable to determine the statistical significance of these results because of the small number of samples and the impossibility of absolute exclusion of cysticercosis.

Our results show that, on the whole, no test has a statistical advantage over the others. However, the ELISA is significantly more effective than the HAT or the FAT in detecting active cysticercosis when CSF samples are tested (P = 0.02). This difference may be related to the small number of samples, although with an agreement of only 33% between the ELISA and the FAT this seems unlikely.

When selecting a test system there are other aspects to be considered; for example, although the HAT does not require sophisticated equipment, interpretation of the results is subjective and requires a skilled operator. The FAT has the disadvantages of both requiring expensive equipment and needing an experienced technician to interpret the result. An examination of Fig. 1 shows that with both the HAT and the FAT a number of results fall within the area which could easily be misinterpreted. Although the ELISA may not be any more accurate than the other tests, the result is always unequivocal and cannot be misinterpreted through inexperience.

It was not the intention of this study to promote any particular technique but rather to assess the currently available tests. It can be concluded that the tests are highly effective in detecting active cysticercosis (except when the FAT is used on a CSF sample) and that a positive result, especially if produced by more than one test, can be regarded with a high degree of certainty as indicating cysticercosis.

We wish to thank the staff of the Cato Ridge abattoir for supplying the infected pork, Professor J. van Dellen and Dr M. Walshman of the Departments of Neurosurgery and Radiology at Wentworth Hospital for providing the serum and CSF samples and CT scan results, and the South African Medical Research Council for permission to publish.
Endogenous immunoreactive digitalis-like substance in neonatal serum and placental extracts

A. D. BEYERS, L. L. SPRUYT, H. I. SEIFART, A. KRIEGLER, D. P. PARKIN, P. P. VAN JAARSVELD

Summary

The therapeutic levels of digoxin in the serum of untreated neonates delivered to mothers who had not received the drug prenatally were detected by radio-immunoassay. Digoxin levels in neonates should be interpreted with caution because of the unknown contribution by the endogenous digitalis-like substance (DLS) to the level of the drug.

Three commercially available radio-immunoassay kits were compared with regard to their sensitivity and reproducibility in detecting the endogenous DLS. The kit from Commercial Assays (Cambridge, Mass., USA) was selected for further investigations. In a series of 35 paired samples of maternal and cord blood the average DLS values in terms of digoxin were 0.57 ± 0.07 and 0.81 ± 0.27 ng/ml respectively. This difference is statistically highly significant. In the case of infants with DLS values of 1 - 1.5 ng/ml in terms of digoxin, approximately 1 week was required to reach therapeutic digoxin levels, i.e. below 0.5 ng/ml.

Gel chromatography showed that the DLS in neonatal serum was more closely associated with protein than is authentic digoxin. In placental extracts it followed the elution profile of the protein completely, but it shifted to fractions with a lower molecular weight than haemoglobin after trypsinization. The level of DLS in neonatal serum was also increased by more than half its original value by trypsinization. Proteolysis therefore seems to have a releasing effect on DLS. The molecular size of this substance is probably in the same range as that of polypeptides, since it was not dialysable from their high-affinity inhibitory action on Na+-K+-adenosine

REFERENCES