Hydatidosis Diagnosis by Double Diffusion in Agar with Arc 5 Detection

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To further assess the double diffusion arc 5 (DD5) test for human hydatidosis, the test was performed on sera from 45 patients with surgically confirmed hydatidosis and 54 with other pathologies in Santiago, Chile. Combined with tests reported previously, this brought the total number of study subjects with confirmed hydatidosis to 98 and the total with other pathologies to 128. Overall, the test yielded positive results with sera from 55 (56%) of the 98 serologically confirmed cases, the degree of seropositivity among patients with pulmonary hydatidosis (36%) being relatively low.

The criteria adopted for a positive result were the presence of arc 5 or, in the absence of arc 5, the presence of three or more bands of precipitate. On the basis of these criteria, no false positive results were detected using sera from the 128 patients with other pathologies. It is concluded that using the DD5 test to diagnose hydatidosis in clinically selected individuals can provide results of high specificity; i.e., positive results can be taken as confirming presence of the infection. On the other hand, a negative DD5 result does not constitute definite proof that no hydatid cysts are present.

Hydatidosis is considered one of the principal parasitic zoonoses affecting man in Chile, the estimated prevalence being 200 cases per 100,000 inhabitants. Annually, the average toll is about 800 new hospitalized cases and 60 deaths (1-2).

Clinical diagnosis of the disease is facilitated by combining various techniques—radiography, ultrasound scanning, scintigraphy, computerized axial tomography, and in vitro detection of antibodies against Echinococcus granulosus (3).

Coltorti and Varela-Díaz described the double diffusion in agar reaction with arc 5 detection (DD5) in 1978. This test can be used for immunologic confirmation of hydatid infection (4).

The simplicity of the DD5 technology means that hospital laboratories in outlying or endemic areas can avail themselves of an examination that is easy to perform. However, the freeze-dried antigen, like the control antiserum for DD5 testing, has to be provided by a specialized center. In Chile, this function is performed by the Public Health Institute (Instituto de Salud Pública, ISP), through the Parasitology Reference Laboratory in Santiago.

We have been using DD5 routinely in our laboratory since 1980 (see photo) for clinical and epidemiologic serodiagnosis (5-6). In a previous communication (5), we examined the sensitivity and specificity of DD5 to sera from 53 patients with...
Two DD5 test slides showing positive and negative results. In the upper (negative) slide, only the control (small circle) produced a band of precipitate. In the lower (positive) slide, the test serum in the large circles produced two arc 5 bands coincident with the control band.

hydatidosis and from 74 with other pathologies.

In view of DD5’s importance as a diagnostic test for this parasitosis in Chile, we decided to complement the earlier case records by testing 45 new surgically confirmed patients with hydatidosis and 54 patients with other pathologies, and also to reanalyze the performance of DD5 in accordance with the anatomic location and biological state of the hydatid cyst in a total of 98 patients with surgically confirmed cases of hydatidosis.

MATERIALS AND METHODS

We reviewed the clinical histories of 226 patients with symptomatology suggesting hydatidosis who were admitted to the San Juan de Dios Hospital in Santiago between 1980 and 1986. In 98 cases the diagnosis was confirmed by surgery, while in the other 128 the final diagnoses were neoplasias at various sites, benign tumors, cirrhosis of the liver, and other ailments. The ages of the male and female patients with hydatidosis ranged from 4 to 89 years, the average age being 37. The nonhydatid patients’ ages ranged from 4 to 81 years, the average age being 45.

The DD5 test was performed as described by Coltorti and Varela-Díaz (4). The freeze-dried antigen and control antiserum used in the test were supplied by the ISP. The test was considered positive when a band of precipitate identical to the control band corresponding to arc 5 was observed; or, if arc 5 were absent, when three or more precipitation bands were observed (7).

RESULTS

Of the combined total of 98 surgically confirmed hydatidosis cases, 55 yielded positive results (Table 1). Sera from
Table 1. Results of the DD5 test performed with sera from 98 patients with surgically confirmed hydatidosis cases according to the site of the hydatid cyst.

<table>
<thead>
<tr>
<th>Cyst site</th>
<th>No. of patients</th>
<th>Positive results</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>43</td>
<td>27</td>
<td>62.8</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>25</td>
<td>9</td>
<td>36.0</td>
<td></td>
</tr>
<tr>
<td>Multiple (more than one organ)</td>
<td>17</td>
<td>11</td>
<td>64.7</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>3</td>
<td>1</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>7</td>
<td>70.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>55</td>
<td>56.1</td>
<td></td>
</tr>
</tbody>
</table>

62.8% of the patients with single or multiple hepatic cysts yielded positive responses, as did sera from 64.7% of the patients with cysts in more than one organ. On the other hand, only nine of 25 pulmonary hydatidosis cases (36%) and only one of three renal hydatidosis cases (33%) yielded positive results. Among the patients with cysts at other sites, seven of 10 (70%) were DD5 positive; of these, four had retroperitoneal cysts, two had osseous hydatidosis, and one had thyroid gland hydatidosis.

Sera from another five of the confirmed hydatidosis cases (four with hepatic hydatidosis and one with pulmonary hydatidosis) yielded one or two bands of precipitate without the presence of arc 5; in accordance with the positivity criteria established for the test, this result was deemed negative.

Table 2. DD5 test results obtained with sera from 55 patients with confirmed hydatidosis, according to the site and whether the hydatid cyst was simple (hyaline) or complicated (broken, infected, fistulized, etc.).

<table>
<thead>
<tr>
<th>Site and biological condition of cyst</th>
<th>No. of patients</th>
<th>Positive results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Simple</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Complicated</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Simple</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Complicated</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>26</td>
</tr>
</tbody>
</table>

All 128 patients with nonhydatid pathologies yielded negative DD5 results. In one case, where the patient had an ovarian cystoma, the serum yielded a band of precipitate without the presence of arc 5; in accordance with the positivity criteria established for the test, this result was deemed negative.

DISCUSSION AND CONCLUSIONS

Our findings indicate that the DD5 test has low sensitivity to pulmonary hydatidosis, which tends to confirm existing knowledge regarding other serologic tests for cysts at this site (8-9). Among the 98 patients with surgically confirmed hydatidosis, only nine of 25 (36%) with pulmonary cysts were detected with DD5. In contrast, 63% of those with hepatic cysts yielded positive results, a percentage comparable to the 65% positivity attained with patients having cysts in more than one organ. (This finding of greater sensitivity for patients with hydatid cysts in more than one organ has previously been reported—9.)

The presence of complicated cysts that might have been more immunogenic than simple ones did not enhance the observed sensitivity of DD5 to cases of he-
patic hydatidosis. In pulmonary hydatidosis, on the other hand, a positive correlation was observed between complicated cysts and positive DD5 results.

As has been shown previously (4–7), the specificity of the DD5 test for hydatidosis vis-à-vis other nonparasitic ailments proved to be high (100%). Indeed, the presence of arc 5 in the DD5 test would appear to provide immunologic confirmation of hydatid infection. However, this specificity is not absolute, since cross reactions with Echinococcus multilocularis, E. vogeli, and Cysticercus cellulosae have been described; a differential diagnosis is therefore needed, by means of additional examinations, in geographic areas where these other parasites are prevalent (7). In addition, detection of one or two bands of precipitate without the presence of arc 5 is not necessarily indicative of hydatidosis, since such results can be obtained from patients with other afflictions.

In general, the result of the DD5 test is influenced by host-parasite factors including the cyst site(s), the number of cysts present, and possibly the E. granulosus strain involved (9–10).

Overall, the sensitivity and specificity of DD5 demonstrated in this study suggest that the positive predictive value of the test for diagnostic purposes (i.e., in a clinically selected sample) is around 99.1%. On the other hand, the negative predictive value (considering a theoretical percentage of false positives close to 0) is only around 73.9%.

In practice, therefore, applying DD5 in routine serologic diagnosis of hydatidosis provides a test with high specificity; i.e., positive results confirm the presence of the infection. However, negative results do not clearly prove the absence of hydatid cysts—especially in pulmonary cases, where DD5 sensitivity is relatively low.

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REFERENCES