Effect of Treatment on Serum Antibody to Hymenolepis nana
Detected by Enzyme-Linked Immunosorbent Assay

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An enzyme-linked immunosorbent assay (ELISA) was developed to measure serum immunoglobulin G
antibodies in 65 patients infected with Hymenolepis nana and 30 noninfected patients. Antibody was detected
in 51 of 65 (sensitivity, 79%) and 5 of 30 H. nana-negative patients (specificity, 83%). Nine patients infected
with H. nana were treated with praziquantel (20 to 25 mg/kg of body weight). Antibody disappeared from the
sera at 90 days in six patients, five of whom had eliminated H. nana. Antibody persisted in three patients in
whom H. nana infection did not clear after treatment. The H. nana ELISA had a high rate of cross-reactions
with sera from patients with cysticercosis (8 of 29 [28%]) and hydatidosis (8 of 23 [35%]). The ELISA for H. nana
may be useful for defining the epidemiology of H. nana infections, especially in areas free from
cysticercosis and hydatidosis.

Hymenolepis nana is a tapeworm that commonly infects both human beings and rodents. It has a worldwide
distribution and is especially prevalent in tropical regions (10). It was the most common helminth detected in stool examinations
in a survey in a Peruvian urban shanty town (2, 9).

SeroLOGY FOR H. nana HAS BEEN PERFORMED IN ANIMALS
infected with this parasite (11–13), but no study has yet demonstrated the presence of antibodies in infected humans.
We developed an enzyme-linked immunosorbent assay (ELISA), using the soluble portion of a homogenate of adult
H. nana worms. This assay was then used to determine the relationship between H. nana infection and the presence
of antibodies. We also examined whether the ELISA might prove useful as a diagnostic option for the evaluation of
therapy for H. nana infection.

Sera were obtained from outpatients presenting to the Rimac and Maria Auxiliadora Hospital, both of which serve
shanty town areas of Lima, Peru. Sera from 65 Peruvian patients (median age, 10 years; range, 3 to 44 years) with H. nana
egative examination were used as controls in the ELISA for H. nana. Sera from 23 tissue-confirm positive sera (median age, 15
months, 38 years) of hydatidosis (Echinococcus granulosus) and 29 tissue-confirm negative sera (median age, 30 years) of cysticercosis (Taenia solium) were examined to determine the rate of
cross-reaction that occurred in the ELISA for H. nana. All patients with cysticercosis had at least one stool examination
negative for H. nana.

Of the 65 patients infected with H. nana described above, 9 were treated with a single dose (20 to 25 mg/kg of body
weight) of praziquantel (Bayer) (3) and had serial stool and serum (3 months after therapy) specimens available for
testing.

Stool specimens were emulsified in Merthiolate-iodine-
Formalin and were examined on direct wet smear after they
were concentrated with Formalin-ether (1, 7).

Adult worms of H. nana (human subspecies) were collected from the small intestines of infected mice. Worms
were homogenized in physiological phosphate-buffered saline (pH 7.4) at 4°C and then sonicated four times at 30 KHz
for 5 min continuously, after which the suspension was centrifuged at 4°C at 8,000 × g for 30 min. The supernatant
was collected, divided into aliquots, and maintained frozen at −70°C until use. The protein content was determined by the
method of Bradford (4).

The polystyrene microtitration plates (Immulon 1; Dynatech Laboratories, Chantilly, Va.) were left overnight
with 100 μl of antigen (1 μg of protein per ml) diluted in 0.06 M carbonate buffer (pH 9.6) at 4°C. Excess antigen was
removed by washing the wells with phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20. Unbound polystyrene
binding sites were blocked by incubating the plates for 1 h at 37°C with 1% skimmed milk diluted in phosphate-
buffered saline containing 0.05% Tween 20. The wells were washed, and a 100-μl volume of the serial serum dilution
(1:100, 1:500, and 1:2,500) was added to duplicate wells and then incubated for 1 h at 37°C. Three negative and one
positive serum specimen were used as controls on each plate. After washing, goat anti-human immunoglobulin G
(IgG) conjugated with peroxidase (1:1,000; Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was added and
incubation was done for 1 h at 37°C. The microplates were washed, and 100 μl of substrate consisting of 10 μl of
peroxide and 4 μg of o-phenylenediamine per 10 ml of citrate buffer (0.1 M citric acid, 0.1 M sodium citrate) was added
after the plates were incubated for 10 to 15 min at room temperature. The reaction was stopped by adding 25 μl of 1
N sulfuric acid and was read at 490 nm in an enzyme immunoassay reader (Titertek Multiskan; Flow Laborato-
ries, McLean, Va.) (8).

The cutoff point and best dilution of antigen were determined by constructing sensitivity and specificity response
tables and selecting the point at which the optimum specificity and sensitivity were achieved. The method has been

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TABLE 1. Serology of nine *H. nana*-infected patients 3 months after treatment with praziquantel

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Presence of <em>H. nana</em> in patient stools</th>
<th>Optical density in ELISA at a 1/100 dilutiona</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretreatment (0 days)</td>
<td>Posttreatment (&gt; 90 days)</td>
</tr>
<tr>
<td>11</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>44</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>+++</td>
<td>+ +</td>
</tr>
<tr>
<td>4</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>30</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>++</td>
<td>–</td>
</tr>
</tbody>
</table>

a There was a significant difference in the optical densities in sera from patients prior to and after therapy (sign test, \( P < 0.0039 \)). There was also a significant difference in seropositivity between patients who were no longer infected with *H. nana* and patients who were still infected (the Fisher test, \( P < 0.001 \)).

b An optical density cutoff point of <0.386 was considered a negative ELISA value.

reported elsewhere (6). In the *H. nana* ELISA, a dilution of 1/100 at an optical density of 0.386 was optimum.

A sensitivity of 79% and a specificity of 83% were obtained for confirmed positive and negative cases. There was a high rate of cross-reaction in the *H. nana* ELISA when tested with sera from patients with hydatidosis and cysticercosis. Sera from 35% (8 of 23) of the patients with hydatidosis and 28% (8 of 29) of the patients with cysticercosis gave false-positive responses.

Of the nine symptomatic patients with *H. nana* that were treated with praziquantel, five had no ova present in their stool specimens 90 days after treatment. Antibodies were not detected by ELISA in any of these five patients after therapy. Of the four patients who, after treatment, were still excreting *H. nana* ova, antibodies were still detected by ELISA in three of them, but at lower levels compared with the pretreatment optical density values. The other patient's optical density values did not change from pretreatment levels (Table 1).

Nearly all individuals infected with *H. nana* had IgG antibodies to this parasite. After effective therapy the antibodies to *H. nana* disappeared. Although the antibody response to *H. nana* in humans has not been described previously, its presence in mice infected with *H. nana* is well known (11).

Immunoblotting has demonstrated that some of the protein bands found in adult *H. nana* worms are also present in the crude extracts of cystic hydatid *E. granulosus* and *T. solium* larval forms. These cross-reacting proteins are probably responsible for the high rates of false-positive results obtained in patients with hydatidosis and cysticercosis.

Patients treated for *H. nana* infection rapidly lost their antibody to the parasite. In dogs infected with *Taenia ovis*, there is also a rapid decrease in antibody titer after treatment with praziquantel (9).

Results of the present study suggest that the ELISA may also be a useful tool for demonstrating the epidemiology of *H. nana* infections in areas where cysticercosis and hydatidosis are rarely encountered. Serology with the ELISA will provide a simple, objective, although indirect, method for determining the presence of *H. nana* infections and may assist in the evaluation of drug therapy for this parasite. This is especially important, since ova of *H. nana* may be excreted erratically (5).

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REFERENCES

6. Cysticercosis Group in Peru. Submitted for publication.