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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* eggs in their stools and 30 patients (median age, 15 years; range 9 to 40 years) with stool-negative examinations were studied. Sera from the 30 patients with a single stool-negative examination were used as controls in the ELISA for *H. nana*. Sera from 23 tissue-confirmed cases (median age, 38 years) of hydatidosis (*Echinococcus granulosus*) and 29 tissue-confirmed cases (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 supernant 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 I; 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 mg 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 Laboratories, 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

Age (yr)	Presence of *H. nana* in patient stools		Optical density in ELISA at a 1/100 dilution[a]	
	Pretreatment (0 days)	Posttreatment (>90 days)	Pretreatment	Posttreatment
11	+++	+	1.072	0.572
19	+++	+	0.832	0.384[b]
44	+	−	0.817	0.294[b]
6	+++	+++	0.439	0.572
4	+++	−	0.634	0.300[b]
5	++	+++	0.644	0.485
8	+	−	0.804	0.300[b]
30	+++	−	0.489	0.169[b]
9	++	−	0.576	0.302[b]

[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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