A DOT-ELISA TEST USING MONOCLONAL ANTIBODY-PURIFIED ANTIGENS FOR THE DIAGNOSIS OF PARAGONIMIASIS CAUSED BY *PARAGONIMUS HETEROTREMUS*

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Abstract. A dot enzyme-linked immunosorbent assay (dot-ELISA) using antigens purified by monoclonal antibody-affinity chromatography was developed for detecting antibodies to *Paragonimus heterotremus* in four groups of subjects. They consisted of 30 patients with *P. heterotremus* infection, 93 patients with other parasitic infections, 18 patients with pulmonary tuberculosis and 30 normal, healthy controls. Sensitivity, specificity, as well as positive and negative predictive values of the test were 100, 97, 88, and 100%, respectively.

INTRODUCTION

Lung fluke infection, paragonimiasis, is an important public health problem in many parts of the world, including Asia, South America, and Africa. *Paragonimus westermani* is the species well recognized as a causative agent of pulmonary paragonimiasis in East Asia, however, this fluke is rarely seen in Southeast Asia. Rather another species, *P. heterotremus* has been reported to cause human paragonimiasis in Thailand and Lao PDR (Miyazaki and Harinasuta, 1966; Miyazaki and Fontan, 1970; Vanijanonta *et al*, 1981). Immunodiagnostic tests to detect antibodies against *P. heterotremus* play a supplementary role to the routine parasitological methods for diagnosis of the infection. In addition, they specially help in detecting ectopic paragonimiasis as well as early infection.

Previously, it has been reported that a 31.5 kDa component of the excretory-secretory (ES) products of adult *P. heterotremus* was a specific antigen for diagnosis of human infection (Maleewong *et al*, 1992). Furthermore, monoclonal antibody (MAb) against the 31.5 and 22 kDa components of the ES antigens has been produced and used in affinity chromatography for the isolation of the specific components from the crude antigens. These affinity purified antigens so obtained were used in an indirect ELISA and found to give high sensitivity and specificity (Maleewong *et al*, 1997). The standard indirect ELISA, however, are less practical for field surveys. Another assay, dot-ELISA has been applied successfully for screening antibodies to different parasitic infections (Pappas *et al*, 1984; Kumar *et al*, 1985; Londner *et al*, 1987; Shaheen *et al*, 1989; Su and Prestwood, 1991). In this report, we describe the dot-ELISA using the purified antigens for the serodiagnosis of paragonimiasis.

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MATERIALS AND METHODS

Antigen preparations

The crude ES antigens of *P. heterotremus* adult worms were prepared as previously described (Maleewong *et al*, 1997). The specific antigens were isolated from the crude antigens by affinity chromatography using MAb 10F2-conjugated Sepharose column as previously described (Maleewong *et al*, 1997). SDS-PAGE and Coomassie brilliant blue staining of the eluted fraction revealed two protein bands with molecular mass of the 31.5

and 22 kDa, respectively. These antigens were used for antibody detection by dot-ELISA.

Sera

Thirty paragonimiasis sera were obtained from villagers in the endemic area of Thailand whose sputa contained *P. heterotremus* eggs. All of these patients had a history of eating raw crabs from mountainous stream between 3 and 6 months prior to blood collections. Their clinical symptoms consisted of bronchitis with gelatinous, tenacious, rust-brown pneumonic like golden fakes and blood-streaked sputum. Sera from patients with opisthorchiasis, opisthorchiasis with other parasitic infection, gnathostomiasis, angiostrongyliasis, trichinosis, cysticercosis, schistosomiasis, capillariasis and fascioliasis were obtained from either parasitologically confirmed cases or cases whose stool examination reveal specific helminth eggs or larvae. Tuberculosis sera were obtained from patients whose clinical findings were compatible with pulmonary tuberculosis and their sputa were positive for acid fast tubercle bacilli. Negative control sera were obtained from healthy adults residing in areas in which paragonimiasis was not present. Stool examinations were done using the formalin-ether concentration method (Erdmann, 1981).

Dot-ELISA

The optimum concentration of the purified antigens, 540 ng/ml, was previously determined by titration and used throughout the experiment. Two microlitres of the antigens in 0.1 M phosphate-buffered saline solutions (PBS), pH 7.5, were spotted separately on a 6 × 12 mm nitrocellulose paper strip (NC) (Hoefer, Pharmacia Biotech, USA) and air-dried for 30 minutes. The unoccupied sites of NC were then blocked with 0.1 M PBS, pH 7.5, containing 5% skim milk for 30 minutes. After blocking, the NC were incubated with 500 µl of human sera, diluted 1 : 100 in blocking buffer, for 2 hours at room temperature with gentle shaking. The strips were washed five times with fresh blocking solution and subsequently incubated with 500 µl goat anti-human IgG peroxidase conjugate (Cappel, Organon Teknika corporation, Cappel™ Research product, North Carolina, USA), diluted 1 : 1,000 in blocking buffer for 2 hours at room temperature. After washing, the strips were developed in 3, 3-diaminobenzidine-tetrahydrochloride solution. The reaction was stopped at 5 minutes by washing the strips with distilled water. Results, an appearance of brownish dots, were observed with the naked eye and recorded arbitrarily according to colored intensity as +, ++, +++ and ++++, each in the order escalated. The precision of the dot-ELISA was also investigated by performing the test on different days by using the same pooled positive serum, the same batch of antigens, and the same conditions. Identical results obtained from all of the tests indicating that day to day variation was minimal. Sensitivity, specificity and predictive values were also calculated (Galen, 1980).

RESULTS

The reaction of +++ was used as the cut-off limit to distinguish positive and negative sera and the results of the dot-ELISA using purified antigens of *Paragonimus heterotremus* crude ES antigens are summarized in the Table 1. While the sensitivity of the test was 100%, *ie* all paragonimiasis sera were

Table 1

Sensitivity and specificity of dot-ELISA using purified *Paragonimus heterotremus* antigens for the serodiagnosis of paragonimiasis.

Subjects	No. of positives/total (%)
Paragonimiasis	30/30 (100)
Gnathostomiasis	0/8 (0)
Angiostrongyliasis	0/7 (0)
Cysticercosis	0/7 (0)
Schistosomiasis	0/7 (0)
Capillariasis	0/4 (0)
Trichinosis	0/18 (0)
Fascioliasis	4/7 (57.1)
Opisthorchiasis	0/24 (0)
Opisthorchiasis and other parasitosis*	0/11 (0)
Tuberculosis	0/18 (0)
Healthy controls	0/30 (0)

* Total of 11 cases, six have *Opisthorchis* and hookworm, three have *Opisthorchis*, hookworm and minute intestinal flukes and two subjects have *Opisthorchis* and *Trichuris*.

positive, the specificity was found to be 97% as a result of false positive associated with four fascioliasis sera. The positive and negative predictive values of the test were 88% and 100%, respectively.

DISCUSSION

In this study, the dot-ELISA using purified ES antigens gave high sensitivity and specificity. The cross-reactivity was found by the dot ELISA only with sera from patients with fascioliasis, however, no cross reaction was observed by the standard indirect ELISA (Maleewong *et al*, 1997). The dot-ELISA should be carefully interpreted in the endemic area of fascioliasis.

The dot-ELISA has an advantage over the indirect-ELISA in several aspects. Nitrocellulose papers spotted with antigens are stable for at least three months at -20°C (data not shown), all incubation steps are performed at room temperature, and the results can be read with naked eye, thus an expensive spectrophotometer is not required. The test is applicable then to diagnosis in a field as well as in many laboratories which are not well-equipped.

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