COMPARISON OF ADULT SOMATIC AND EXCRETORY-SECRETORY ANTIGENS IN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR SERODIAGNOSIS OF HUMAN INFECTION WITH *FASCIOLA GIGANTICA*

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Abstract. Adult somatic antigen extract of *Fasciola gigantica* was compared with excretory-secretory (ES) antigen in an enzyme-linked immunosorbent assay (ELISA) for serodiagnosis of human fascioliasis gigantica. The absorbance values in ELISA using the adult somatic antigen were not significantly different from the values obtaining using ES antigen ($p > 0.05$). The diagnostic sensitivity, specificity and positive and negative predictive values of the test using adult somatic extract as antigen were 100%, 98%, 70% and 100%, respectively. On the other hand, these values of the test using adult ES antigen were 100%, 99.3%, 87.5% and 100%, respectively. It appears that both somatic and ES antigens are effective antigens for use in the serodiagnosis of human fascioliasis gigantica.

INTRODUCTION

Fascioliasis caused by *Fasciola gigantica* is one of the major public health problems in Thailand (Tesana *et al*, 1989). It is a primarily disease of ruminants. Humans are usually infected by the ingestion of aquatic plants that contain the infected metacercariae. Diagnosis of this liver fluke infection is based on clinical features as well as laboratory tests. The most reliable means is the finding of *Fasciola* eggs in the stool of infected individuals. However, the detection of eggs in stool is an insensitive method and is reliable in hands of experienced personnel. The diagnosis is even more difficult in mixed parasitic infections, as *Fasciola* eggs are difficult to distinguish from those of intestinal flukes such as *Fasciolopsis buski* and *Echinostoma* spp. For these reasons, serology plays a supplementary role to parasitological methods. Moreover, immunological tests become the most practical useful and dependable assays in ectopic fascioliasis caused by aberrant migration of the worms or during the lag period between initial infection with metacercariae and sexual maturation of the egg-laying adult worms.

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Recent studies in human and animal fascioliasis caused by *F. hepatica* suggest that an enzyme-linked immunosorbent assay (ELISA) is a useful serodiagnostic test (Espino *et al*, 1987; Rivera Marrero *et al*, 1988; Santiago and Hillyer, 1988; Sinclair and Wassall, 1988; Espino *et al*, 1992). However, the evaluation of serological tests for the immunodiagnosis of patients infected with *F. gigantica* is still lacking. In addition, the effectiveness of *F. gigantica* adult worm somatic or excretory-secretory (ES) antigens as antigens in ELISA have not been studied.

The purpose of this investigation was to compare a somatic extract of *F. gigantica* with the ES antigen for use in ELISA for serodiagnosis of human fascioliasis gigantica.

MATERIALS AND METHODS

Parasite

Adult *F. gigantica* were obtained from infected bovine livers collected from Khon Kaen Province, Thailand. The worms were washed with sterile saline and identified as *F. gigantica* according to criteria previously described (Watanabe, 1965; Sahba *et al*, 1972).

Antigens

Adult *F. gigantica* somatic antigen was prepared by homogenization and extraction as described by Maleewong *et al* (1992) with some modifications. Briefly, the adult worms were homogenized with a tissue grinder in a small volume of 0.1 M PBS pH 7.4 containing 0.1 mM of phenylmethylsulfonyl fluoride (PMSF), 0.1 mM of tosylamide-2-phenylethyl-chloromethyl ketone (TPCK) and 1 μM of N-(N-[L-3-trans carboxyoxiran-2-carbonyl]-L-leucyl)-agmatine (E-64). The suspension was then sonicated with an ultrasonic disintegrator and centrifuged at 10,000*g* for 30 minutes at 4°C. The supernatant was used as the source of antigen. Protein content was estimated by the standard method (Lowry *et al*, 1951).

Adult *F. gigantica* ES antigen was prepared from spent culture medium [RPMI 1640 (Gibco, USA] containing 0.1 mM PMSF, 0.1 mM TPCK, 1 μM E-64, 100 U/ml penicillin G and 100 μg/ml streptomycin in which the worms had been maintained for 6 hours at 37°C under 5% CO_2 in air. After incubation, the worms were removed and the collected spent medium was centrifuged 10,000*g* at 4°C for 30 minutes. The medium was concentrated by ultrafiltration using Amicon YM3 membrane filter (Grace Co, USA) and dialyzed against distilled water containing the same proteinase inhibitors. The protein content of this ES was also determined by the same method as above (Lowry *et al*, 1951).

Sera

Seven fascioliasis sera were obtained from parasitologically confirmed human cases of *F. gigantica* infection, all were selected on the basis of the removing of *F. gigantica* adult worms during cholecystectomy and T-tube choledochostomy or bile ducts operation. These sera were collected from the hospitals in the endemic area (Khon Kaen and Udorn Thani Provinces) of northeastern Thailand.

To examine potential cross reactivity, serum samples obtained from individuals with various parasitic diseases other than fascioliasis were used. These samples were obtained from parasitologically confirmed cases of gnathostomiasis, angiostrongyliasis, cysticercosis, schistosomiasis japonicum, capillariasis, paragonimiasis heterotremus and opisthorchiasis. Other parasitosis sera were from cases who were positive for parasites or their products. Of 17 other hepatobiliary disease sera were also included.

Negative control sera were from apparently healthy volunteers. Examination of their stools at the time of blood collection gave no evidence of any intestinal parasitic infection. Pooled positive reference serum was prepared by combining equal volumes of each fascioliasis serum. Pooled negative reference serum was prepared similarly from negative control sera.

Enzyme-linked immunosorbent assay

The solid phase ELISA was performed in a microtiter plate as described by Voller *et al* (1976). Optimum conditions were determined by a chequer board titration against pooled positive and negative reference sera. The optimum antigen concentrations were the 0.5 and 1 μg/wells for ES and somatic antigens, respectively. The enzyme conjugate used was peroxidase-conjugated goat antihuman immunoglobulin G (Cappel Laboratory, USA).

Sera were tested at 1 : 200 dilution. The positive reference serum was included in each microtiter plate for correction of day to day variation. Test sera which gave an absorbance reading (at 490 nm) greater than the mean plus 3 standard deviation of the negative control sera were considered as positive.

Statistical analysis

The sensitivity, specificity and the predictive values were calculated using the method of Galen (1980). The statistical analysis significance was performed using Student's *t* test and Mann-Whitney *U* test, as appropriate.

RESULTS

The absorbance values of fascioliasis and other sera in this ELISA are summarized in the Table 1. The values obtained by using somatic antigen were not significantly different from those using ES antigen ($p > 0.05$). The sensitivity of this ELISA, determined by the positivity of confirmed fascioliasis sera, was 100% with either somatic or ES

Table 1

Absorbance values of various sera in enzyme-linked immunosorbent assay using somatic and excretory-secretory antigens.

Sera	No. tested	Somatic antigens		ES antigen	
		Av[a]	No. positive	Av[a]	No. positive
Fascioliasis	7	0.942 ± 0.243	7	1.182 ± 0.246	7
Gnathostomiasis	9	0.082 ± 0.057	0	0.085 ± 0.055	0
Angiostrongyliasis	8	0.053 ± 0.047	0	0.055 ± 0.048	0
Cysticercosis	7	0.067 ± 0.072	1	0.095 ± 0.096	1
Schistosomiasis	7	0.028 ± 0.018	0	0.039 ± 0.022	0
Capillariasis	4	0.041 ± 0.026	0	0.042 ± 0.032	0
Paragonimiasis	32	0.072 ± 0.040	0	0.075 ± 0.037	0
Opisthorchiasis	24	0.041 ± 0.022	0	0.053 ± 0.027	0
Mixed helminthiasis[b]	10	0.064 ± 0.029	0	0.066 ± 0.025	0
Hepatobiliary diseases[c]	17	0.131 ± 0.080	2	0.092 ± 0.024	0
Healthy control	30	0.048 ± 0.062	0	0.040 ± 0.052	0

[a] Absorbance value (490 nm).
[b] Total of 10 cases, 6 had *Opisthorchis viverrini* and hookworm infections, and 4 had mixed infection of 4 helminths including hookworm, *Strongyloides stercoralis, Trichuris trichiura* and minute intestinal flukes.
[c] Total of 17 cases, 10 were cholangiocarcinoma, 4 were hepatoma and 3 were viral hepatitis.

antigens (Table 1). The specificity of the ELISA for *Fasciola* infection was 98% using somatic antigen and 99.3% using ES antigen. In the cysticercosis group, 1 of 7 cases gave a false positive result with both antigens. One sample of cholangiocarcinoma and 1 of viral hepatitis sera taken from the other hepatobiliary diseases group gave a false positive with low reaction only for somatic antigen. The positive and negative predictive values of the test using somatic antigen were 70% and 100%, respectively. On the other hand, these values of this test using ES antigen approached 87.5% and 100%, respectively.

DISCUSSION

It has been demonstrated that adult somatic and ES antigen are an attractive choice for use in an ELISA for the diagnosis of *F. hepatica* infection (Espino *et al*, 1987; Rivera Marrero *et al*, 1988; Santiago and Hillyer, 1988; Sinclair and Wassall, 1988; Espino *et al*, 1992). The present study showed that an ELISA using adult *F. gigantica* ES antigen has the same sensitivity and specificity as somatic antigen for diagnostic purposes. Common antigens may be present in both antigen preparations as demonstrated for *Opisthorchis viverrini* (Wongratanacheewin *et al*, 1988), *Paragonimus heterotremus* (Maleewong *et al*, 1990) and *F. hepatica* (Lehner and Sewell, 1980). Further study is required to identify the source of antigens in the worm and determine the antigenic component of the antigens.

In conclusion, the present study using the ELISA technique is potentially useful for immunodiagnosis of fascioliasis caused by *F. gigantica*. However, the low cross-reactivity with sera from patients with cysticercosis and other hepatobiliary diseases has been shown. This may be due to either cross reactivity or subclinical *Fasciola* infection. Cross-reactivity between *F. gigantica* somatic antigens and some sera from patients infected with other parasitosis has also been demonstrated by Youssef and Mansour (1991). Finally, purification and examination of individual antigens will be required to identify both sensitive and specific antigen for use in the ELISA.

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