THE EVALUATION OF THE 29 AND 31 kDa ANTIGENS IN FEMALE *ANGIOSTRONGYLUS CANTONENSIS* FOR SERODIAGNOSIS OF HUMAN ANGIOSTRONGYLIASIS

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Abstract. The objective of this study was to evaluate the crude somatic extract of female *Angiostrongylus cantonensis* by ELISA and to evaluate the 29 kDa and the 31 kDa components of female worm by EITB for serodiagnosis of human angiostrongyliasis. Using ELISA, cross reaction occurred among all sera tested except capillariasis. The sensitivity, specificity, positive and negative predictive values of ELISA were found to be 100%, 66.8%, 27.1% and 100% respectively. Using EITB, The 29 kDa component cross-reacted with most of the heterologous sera while the 31 kDa component was recognized by 69.2% (18/26) sera from angiostrongyliasis patients, cross-reacted only with trichinellosis, trichuriasis and opisthorchiasis sera. This component may possibly used as reliable agents for angiostrongyliasis out of other tissue infected helminthiasis. The sensitivity, specificity, positive and negative predictive values of EITB for the 31.0 kDa component were 69.2%, 82.4%, 46.2% and 92.5% respectively.

INTRODUCTION

Angiostrongylus cantonensis, the rat lungworm, is known to be a causative agent of human eosinophilic meningitis or eosinophilic meningoencephalitis in Asia and Pacific islands (Alicata and Jindrak, 1970). In Thailand, there are numerous clinical cases of eosinophilic meningoencephalitis relating to the ingestion of fresh water snail, *Pila* spp. (Tangchai *et al*, 1967; Punyagupta *et al*, 1970; Witoopanich *et al*, 1991). The most specific diagnosis is the finding of parasites in the CSF of patients or the brains of autopsied cases, and occasionally in the eyes. Since the finding of parasites is very rare, the immunological technique is a helpful tool for diagnosis. Although various immunodiagnostic techniques have been successfully used to detect antibodies in serum and cerebrospinal fluid utilizing either crude or partially purified adult worm antigens (Welch *et al*, 1980; Chen, 1986) or young adult worm antigen (Tharavanij, 1979; Cross and Chi, 1982; Yen and Chen, 1991) and circulating antigen of third stage larvae in serum (Shih and Chen, 1991), the results were still not satisfactory. However, Akao *et al* (1992) demonstrated that the 29 kDa and 31 kDa antigens from female *A. cantonensis* were probably specific antigens for diagnosis of human angiostrongyliasis. Moreover, Eamsobhana *et al* (1995) found that the 31 kDa component presented both in larvae and adults of *A. cantonensis*. The present study aims to evaluate the crude antigen extract of female *A. cantonensis* by using enzyme-linked immunosorbent assay and the 29 kDa and the 31 kDa components of female worms by using enzyme-linked immunotransblot technique for serodiagnosis of human angiostrongyliasis.

MATERIALS AND METHODS

Preparation of parasite antigen

Adult female *A. cantonensis* were obtained from wistar rats experimentally infected with infective third stage larvae from laboratory infected *Biomphalaria glabrata* for 12 weeks. The intact worms were homogenized with a small amount of alumina paste in triple distilled water until all of the worms were thoroughly broken into very tiny pieces. After removing of alumina by centrifugation at 60*g* for 5 minutes, the supernatant was then recentrifuged at 10,000*g*, at 4°C for 30 minutes. The protein content of crude somatic extract (FAC) was estimated by Folin-phenol method (Lowry *et al*, 1951). The extract was stored at -20°C until use.

Sera

Angiostrongyliasis sera were obtained from 3 parasitological confirmed patients and 23 eosinophilic meningoencephalitis patients who had a his-

tory of eating raw pila snails within one month before the onset of disease. Sera from 109 patients with gnathostomiasis, ascariasis, trichuriasis capillariasis, strongyloidiasis, hookworm infection, taeniasis, sparganosis, echinococcosis, paragonimiasis and opisthorchiasis were obtained from parasitologically confirmed cases while toxocariasis and trichinellosis sera were obtained from clinically and immunologically diagnostic cases. Negative control sera were obtained from 32 healthy persons who had no history of eosinophilic meningitis, migratory swelling and other parasitic infections. The positive control was a pool of sera with equal volumes from 3 parasitologically confirmed angiostrongyliasis cases while the negative control was a pool of sera with equal volumes from healthy persons.

Enzyme-linked immunosorbent assay (ELISA)

Immunoglobulin G was measured by ELISA as described by Anantaphruti (1989) with some modification. According to chequer-board titration against pooled positive and negative sera, the optimum antigen concentration was 5 μg protein/ml whereas the optimum dilutions of sera and peroxidase-conjugated rabbit anti-human immunoglobulin G (Dakopatts, Copenhagen Denmark) were 1 : 800 and 1 : 1,000 respectively. The substrate used was 0.05% para-phenylenediamine in citrate buffer pH 4.5 containing 0.003% H_2O_2. The absorbance was read at 492 nm using Titertek multiskan Plus MkII. A sample was considered positive when its OD was greater than 0.105.

Enzyme-linked immunoelectrotransfer blot technique (EITB)

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing condition was performed on a 13% separating gel according to the method described by Tsang *et al* (1983). The amount of FAC was 20 μg for each 5.0 mm width sample lane. The electrophoresis was performed using a discontinuous buffer system (Laemmli, 1970) with a constant current of 30 mA/slab gel for 3-4 hours. After electrophoresis, the resolved FAC bands were electroblotted to a nitrocellulose membrane under a constant voltage of 45 volts for 18 hours. After blotting, the nitrocellulose sheet was washed 3 times with phosphate buffer saline (0.01 M Na_2HPO_4/NaH_2PO_4, 0.15 M NaCl, pH 7.2) containing 0.1% Tween 20 (PBS-T) with agitation on a rotary rocking (side to side). The sheet was then soaked in a blocking solution (3% Gelatin in PBS-T) for 1 hour with agitation. The sheet was cut into a 4-mm wide strip, washed as above and then exposed to diluted serum (1 : 100 in PBS-T) for 6 hours at room temperature with agitation and 4°C overnight. After washing, the strip was exposed to the peroxidase-conjugated rabbit anti-human immunoglobulin G (Dakopatts, Copenhagen Denmark) at dilution 1 : 1,000 for 3 hours at room temperature with agitation. The strip was washed and incubated with freshly prepared 2, 6 dichlorophenylene indophenol in PBS containing 0.003% H_2O_2. Since the brown bands showed up, the strip was thoroughly rinsed with distilled water and then air dried. Sensitivity, specificity and predictive values were calculated according to the method of Galen (1980).

RESULTS

The crude somatic extract of female *A. cantonensis* (FAC) has been evaluated using ELISA. The absorbance values of angiostrongyliasis and other parasitic infections and normal control sera are summarized in Table 1 and Fig 1. The absorbance values greater than 0.105 which was equivalent to minimum value of the angiostrongyliasis sera was determined as positive. The number of cross-reacting sera to FAC is shown in Table 2. The sensitivity and the specificity of ELISA using FAC were 100% and 66.8% respectively whereas the positive and the negative predictive values were 27.1% and 100% respectively.

EITB is shown in Figs 2 and 3, with the 29.0 kDa and the 31.0 kDa bands reacted with parasitic infected sera. The 29.0 kDa component of FAC was recognized in 3 parasitologically confirmed and 20 suspected angiostrongyliasis sera. Cross reaction with this component occurred among gnathostomiasis (16/20), toxocariasis (4/4), trichinellosis (8/10), trichuriasis (10/10), hookworm infected (9/10), strongyloidiasis (8/9), sparganosis (2/2) and normal control (6/10) sera. The sensitivity, specificity, positive and negative predictive values of this component were 88.5%, 47.1%, 26.7% and 94.9% respectively. Using the same technique, the 31 kDa component of FAC was

Table 1

Absorbance values of homologous and heterologous sera versus crude extract of female *Angiostrongylus cantonensis* using ELISA.

Type of sera	No. of tested sera	Absorbance at 492 nm	
		Range	Mean ± SD
Normal control	32	0.004-0.045	0.022 ± 0.029
Angiostrongyliasis	26	0.105-0.730	0.396 ± 0.215
Gnathostomiasis	20	0.018-0.633	0.207 ± 0.167
Toxocariasis	4	0.060-0.464	0.285 ± 0.145
Ascariasis	5	0.011-0.133	0.060 ± 0.091
Trichuriasis	10	0.141-0.414	0.236 ± 0.080
Trichinellosis	10	0.064-0.336	0.142 ± 0.255
Capillariasis	2	0.021-0.034	0.028 ± 0.007
Hookworm infection	10	0.131-0.366	0.260 ± 0.070
Strongyloidiasis	9	0.087-0.403	0.267 ± 0.395
Taeniasis	15	0.025-0.178	0.101 ± 0.042
Sparganosis	2	0.047-0.287	0.167 ± 0.120
Echinococcosis	2	0.008-0.304	0.156 ± 0.209
Paragonimiasis	10	0.051-0.251	0.157 ± 0.189
Opisthorchiasis	10	0.043-0.469	0.214 ± 0.516

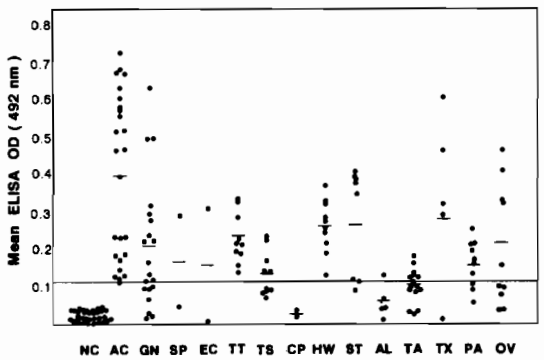

Fig 1–Optical densities of serum ELISA of : NC = Normal control, AC = Angiostrongyliasis, GN = Gnathostomiasis, TX = Toxocariasis, AL = Ascariasis, TT = Trichuriasis, TS = Trichinellosis, CP = Capillariasis, HW = Hookworm infection, ST = Strongyloidiasis, TA = Taeniasis, SP = Sparganosis, EC = Echinococcosis, PA = Paragonimiasis, OV = Opisthorchiasis against crude somatic extract of *Angiostrongylus cantonensis* adult female.

recognized in 3 parasitological confirmed and 15 suspected angiostrongyliasis sera. This component cross-reacted with trichinellosis (8/10), trichuriasis (5/10) and opisthorchiasis (8/10) sera. The sensitivity, specificity, positive and negative predictive values of the 31 kDa component were 69.2%, 82.4%, 46.2% and 92.5% respectively.

DISCUSSION

Identification of a reliable antigenic extract of *Angiostrongylus cantonensis* used for the specific serodiagnosis for human angiostrongyliasis is still controversial (Tharavanij, 1979; Chen, 1986; Yen and Chen, 1991). In present study using FAC antigen, the sensitivity of the ELISA was 100% but the specificity was rather low. Moreover, the cross reaction occurred among gnathostomiasis, toxocariasis, ascariasis, trichuriasis, trichinellosis, hookworm infection, strongyloidiasis, taeniasis, sparganosis, echinococcosis, paragonimiasis and opisthorchiasis. Cross (1978), using ELISA, showed that crude antigen from the brain-stage of *A. cantonensis* cross reacted with sera from hepatic amebiasis, intestinal capillariasis, bancroftian filariasis and schistosomiasis. In addition, Cross (1978) and Chen (1986) demonstrated cross reac-

Table 2

Cross-reaction among other parasitic infected sera against crude extract of female *Angiostrongylus cantonensis* as determined by ELISA.

Type of sera	No. of tested sera	No. of cross-reacted sera	% cross-reaction
Gnathostomiasis	20	13	65
Toxocariasis	4	3	75
Ascariasis	5	1	20
Trichuriasis	10	10	100
Trichinellosis	10	5	50
Capillariasis	2	0	0
Hookworm infection	10	10	100
Strongyloidiasis	9	8	88.9
Taeniasis	15	6	40
Sparganosis	2	1	50
Echinococcosis	2	1	50
Paragonimiasis	10	7	70
Opisthorchiasis	10	5	50

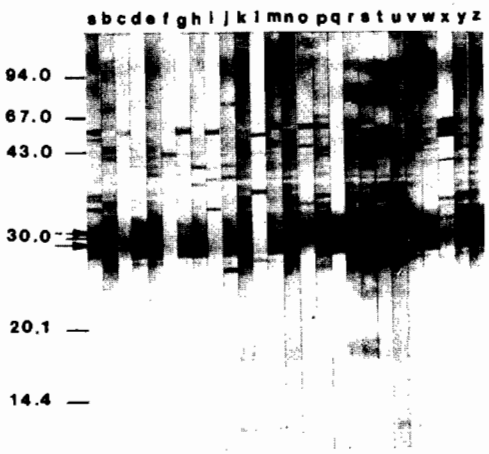

Fig 2–Enzyme-linked immunotransblot of the 29 kDa (→) and the 31 kDa (⇢) components after reacting with 3 parasitologically confirmed (a-c) and 23 suspected angiostrongyliasis sera (d-z).

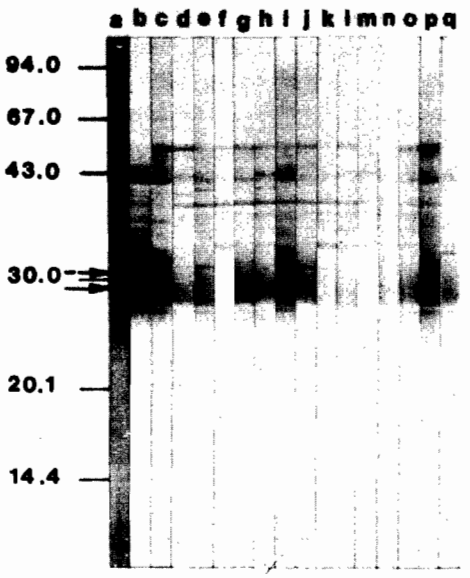

Fig 3–Enzyme-linked immunotransblot of the 29 kDa (→) and the 31 kDa (⇢) components after reacting with 3 parasitologically confirmed angiostrongyliasis (a-c), gnathostomiasis (d), toxocariasis (e), ascariasis (f), trichinellosis (g), hookworm infection (h), strongyloidiasis (i), trichuriasis (j), capillariasis (k), taeniasis (l), sparganosis (m), echinococcosis (n), paragonimiasis (o), opisthorchiasis (p) and normal control (q) sera.

tion among angiostrongyliasis sera and crude antigen preparations of *Entamoeba histolytica*, *Toxoplasma gondii*, *Schistosoma japonicum*, *Dirofilaria immitis* and *Toxocara canis*.

According to EITB, Akao *et al* (1992) suggested that the 29.0 kDa and 31.0 kDa antigenic components were possibly useful for immunodiagnosis of human angiostrongyliasis. Eamsobhana *et al* (1995) also found that the anti-31 kDa component presented in 60% of angiostrongyliasis sera and did not cross-react to other diseases. In comparison with Akao *et al* (1992) and Eamsobhana *et al* (1995), the 29 kDa and the 31 kDa components in this study were not specific for diagnosis of human angiostrongyliasis. However, among these two components the 31 kDa seems to be an interesting immunogen as it does not cross-react with other tissue parasitic infections except trichinellosis, an infection for which definitive diagnosis is available. It is possible that the difference among these three studies is due to the difference in number of sera studied. The higher the number of sera used, the more reliable the evaluation, especially in differentiation among tissue parasitic infections. In conclusion the 31.0 kDa should be further investigated for its reliability for immunodiagnosis of human angiostrongyliasis.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Prof Moriyasu Tsuji, Emeritus Professor of Department of Parasitology, School of Medicine, Hiroshima University for advice and critically reading the manuscript. Sincere thanks are also given to Assoc Prof Jitra Waikagul, Head Department of Helminthology, Faculty of Tropical Medicine, Mahidol University for her invaluable advice, to Miss Hiroko Inoue for her excellent technical guidance, to Miss Nataya Samasanti for her assistance with data processing, to Mr Paron Dekumyoy and Mr Panyawut Hiranyachattada for photographs. Finally, the author is indebted to the General Affairs Department of Hiroshima Prefectural Government, who financed for her visit to Hiroshima University making this study possible.

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