CLINICAL MANIFESTATIONS AND LABORATORY DIAGNOSIS OF EOSINOPHILIC MENINGITIS SYNDROME ASSOCIATED WITH ANGIOSTRONGYLIASIS

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INTRODUCTION

Significant contributions to our understanding of *Angiostrongylus cantonensis* and eosinophilic meningitis both from a historical standpoint and contributions to the clinical and epidemiologic aspects of the disease have been made by workers on the China mainland and Taiwan. Professor H.T. Chen (1935) was the first to describe the parasite after finding it in the lungs of rats in Canton, China and the first human case of angiostrongyliasis was reported by Nomura and Lin (1945) at the Tainan Provincial Hospital in Central Taiwan. They found 10 worms in the spinal fluid of a 15-year-old boy with eosinophilic meningitis who later died. This case was reported in a Japanese journal which had limited distribution and consequently the report was not recognized until the significance of human angiostrongyliasis was brought into prominence by Rosen *et al.*, (1962). After Rosen's publications, physicians on Taiwan became more aware of *Angiostrongylus*-induced eosinophilic meningitis and new cases were reported and studies carried out all aspects of the disease. Taiwan for a period had the distinction of being the only endemic area in Asia where worms were recovered from spinal fluid. In addition, the first report of the recovery of adult worms from human lung was from Taiwan (Yii *et al.*, 1968). Recently Dr. formerly of the Kaohsiung Medical College, published results of his extensive studies in Southern Taiwan and provided a great deal of information on the epidemiological (Yii *et al.*, 1975) and clinical (Yii, 1976) aspects of angiostrongyliasis on Taiwan.

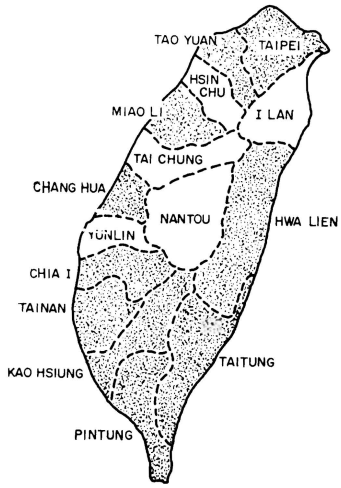

Fig. 1 —Taiwan indicating counties (stippled) where human angiostrongyliasis has been reported. Nearly 250 cases have been reported to September 1977 and *Angiostrongylus cantonensis* recovered from approximately 10%.

To date Taiwan (Fig. 1) has recorded almost 250 cases of eosinophilic meningitis with reports coming from nearly all parts of the island. It is quite possible that twice this number of cases have been seen, but have not been reported. Of these 250 cases *A. cantonensis* has been recovered from about 10%. The rea-

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sons for this comparatively high worm recovery rate on the island are not known, but Yii (1976) suggested that it may possibly be due to the technique used in carrying out spinal taps in Taiwan. In most cases the worms were recovered from children using syringes and 20, 22 or 23 gauge needles. There have been several deaths reported from Taiwan, more than elsewhere, and Dr. Yii feels this may be due to heavier infections.

In most of the cases on Taiwan the patients have eaten or have had contact with the giant African snail, *Achatina fulica.* We have fed *A. fulica* meat sold in Taiwan markets to rats and recovered adult *A. cantonensis* from each pool of uncooked meat. No worms were recovered from rats fed cooked meat, however.

CLINICAL MANIFESTATIONS

The clinical manifestations of eosinophilic meningitis have been adequately covered by Punyagupta (1978) and Table 1 shows some of the major signs and symptoms among the 125 Taiwan cases reported by Yii (1976). During the past year we have had association with a few more cases and additional information is presented herein. Sera from 6 patients diagnosed as having eosinophilic menigitis were sent to NAMRU-2 to test for antibodies to *A. cantonensis.* Three of the cases were confirmed to be *Angiostrongylus*-induced eosinophilic meningitis since *A. cantonensis* larvae were recovered from spinal fluid. No worms were recovered from the other 3 patients and they were considered to be presumptive cases. The symptoms and laboratory findings are shown in Table 2 and were similar to those reported by Yii (1976). In Yii's cases the youngest patient from whom worms were recovered from spinal fluid was 3 years of age while in the present cases, the children were 1½ to 2 years of age; quite young to be eating snails.

Table 1

Major symptoms and clinical signs in cases of eosinophilic meningitis on Taiwan.*

Symptoms	Signs
Headache	Abnormal petellar reflex
Nausea-vomiting	Neck stiffness
Somnolence-lethargy	Abnormal Achilles reflex
Fever	Absence abdominal wall reflex
Constipation	Hepatomegaly
Malaise	Kernig's sign
Anorexia	Abnormal biceps reflex
Cough	Abnormal triceps reflex
Abdominal pain	Eye muscle paralysis

* (From Yii, 1976).

In addition to the cases on Taiwan, 3 other cases of eosinophilic meningitis, presumably caused by *A. cantonensis* were diagnosed. These patients, however, were American.

In October 1976 a group of 15 U.S. Marines were in the wilds of Northern Okinawa, Japan, participating in an exercise in survival training. Under such conditions the men must fend themselves and obtain their own food. In this case some large land snails were found, and a few of the Marines who had previous experience in eating snails on a similar type exercise in Panama, convinced the rest of the group that snails were good eating. Five of the Marines ate only cooked snails, 7 only tasted raw snails out of curiosity, and 3 ate from 5 to 10 raw snails. No ill effects were immediately felt by any of the 15 men and they all departed Okinawa for further training in the Philippines. Approximately 3 weeks after eating the snails the 3 who ate raw snails became ill and were hospitalized. Some of the symptoms experienced by these 3 men are listed in Table 3. All had

Table 2

Symptoms, signs and laboratory findings in six cases of eosinophilic meningitis on Taiwan-1977.

	Positive diagnosis*		Presumptive diagnosis	
Age	1 ½ - 2 years		8 - 12 years	
Sex	2 females, 1 male		1 female, 2 males	
Symptoms and Signs	Fever	3/3	Fever	2/3
	Vomiting	3/3	Vomiting	3/3
	Somnolence	2/3	Stiff neck	3/3
	Eye muscle paralysis	2/3	Headache	2/3
	Anorexia	1/3	Muscle weakness	1/3
	Stiff neck	1/3	Increase tendon reflex	1/3
	Cough	1/3	Malaise	1/3
Laboratory	Eosinophilia	3/3	Eosinophilia	3/3
	CSF-cloudy	2/3	CSF-cloudy	1/3
	Eosinophilic pleocytosis	3/3	Eosinophilic pleocytosis	3/3
	A. cantonensis	3/3	No *A. cantonensis* found	3/3
	Serology-ELISA**	3/3	Serology-ELISA	3/3

*Recovery of *Angiostrongylus cantonensis* larvae in spinal fluid.
**ELISA = Enzyme-linked immunosorbent assay.

Table 3

Symptoms, signs and laboratory finding in three cases of eosinophilic meningitis in U.S. Marines.

Symptoms and signs		Laboratory	
Lower extremity weakness	3/3	Leucocytosis	3/3
Muscle pain	3/3	Eosinophilia	3/3
Flaccid paralysis	2/3	CPK-increase	3/3
Pruritis	3/3	CSF-cloudy	3/3
Stiff neck	2/3	Eosinophilic pleocytosis	3/3
Headache	1/3	Serology - G-D*	1/3
Nausea, vomiting	1/3	CIE**	1/3
Anorexia	1/3	ELISA***	3/3
Diplopia	1/3		
Papilloedema	1/3		
Facial numbness	1/3		

* G-D = Gel-diffusion.
** CIE = Counterimmunoelectrophoresis.
*** ELISA = Enzyme-linked immunosorbent assay.

pruritis, pain and weakness of the lower extremities, 2 had neck stiffness and 2 developed flaccid paralysis. One was more seriously ill with headache, fever, nausea, vomiting, anorexia, papilloedema and diplopia. All had elevated peripheral eosinophils and eosinophilic pleocytosis. The hospital course of 2 was uneventiful but the third required ventricular drainage and repeated lumbar punctures.

It is interesting to note that common symptoms experienced by each of these young Americans were pruritis, muscle pain and weakness of lower extremities. Pruritis and muscle pain are not generally indicated as a symptom of *Angiostrongylus*-induced meningitis, but lower extremity weakness has been reported in number of cases.

Sera and spinal fluid from these men were sent to NAMRU-2, Taipei for testing and the sera for one was found to have to have precipitating antibodies to *A. cantonensis* by gel-diffusion and counterimmunoelectrophoresis. The spinal fluid from this patient was also weakly positive, and oddly enough he was the one with the least amount of disease. The ELISA test was also done on sera from these patients and the results were positive for all.

A. fulica were obtained from the same area on Okinawa where the Marines found the ones that they ate. The snails were examined in our laboratory and a small number of *A. cantonensis* was recovered from 6 of 55 snails. This is a low infection rate compared to Taiwan where most *A. fulica* are infected with large numbers of larvae.

DIAGNOSIS

The diagnosis of the eosinophilic meningitis or eosinophilic meningoencephalitis is primarily presumptive. It is generally based upon clinical findings of meningeal symptoms with eosinophilic pleocytosis. A history of eating or contact with known intermediate or paratenic hosts of the parasite is helpful but not confirmatory.

The definitive diagnosis has been and will continue to be the demonstration of the parasite. *A. cantonensis* has been demonstrated in a number of cases, most notably in spinal fluid from patients in Thailand (Punyagupta, 1978) and Taiwan. Furthermore, in one of Dr. Yii's cases on Taiwan larval and adult worms were recovered at autopsy. The patient was a 5-year-old female who had classical symptoms of the disease and while worms were not recovered from 3 spinal taps, high numbers of eosinophils were seen in each of the taps. The autopsy was carried out approximately 6 hours after death and over 600 worms, some still active, were recovered from the central nervous system (CNS). When lung tissue was examined histologically, sections of worms were found in the pulmonary vessel (Fig. 2) and upon gross examination of the lungs adult worms were found (Fig. 3). Although poorly developed eggs were seen in the uterus of the females neither eggs nor first-stage larvae were found in the lung tissue. Tissue from the CNS were also examined and sections of worms found in the brain (Fig. 4) and cord (Fig. 5) (Yii *et al.*, 1968).

Fig. 2 — Human lung with section of *Angiostrongylus cantonensis* seen in a pulmonary vessel (After Yii *et al.*, 1968).

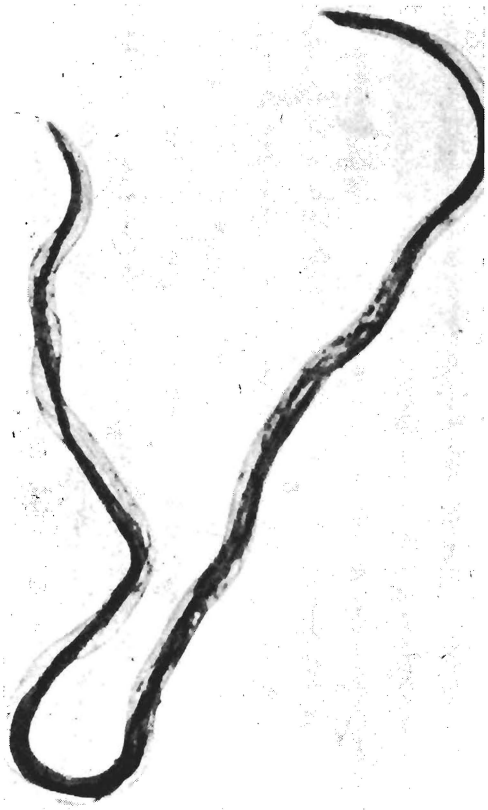

Fig. 3 — Female *Angiostrongylus cantonensis* recovered from the human lung. While eggs were found in the uterus of some females, larvae were not found in lung tissue (After Yii *et al.*, 1968).

Fig. 4 — Human brain with section of *Angiostrongylus cantonensis* in the tissue (After Yii *et al.*, 1968).

Fig. 5 — Human spinal cord with sections of *Angiostrongylus cantonensis* in the tissue (After Yii *et al.*, 1968).

The parasitologic diagnosis of angiostrongylid-induced eosinophilic meningitis is very rare and has been accomplished in only about 50 of the nearly 2,000 presumptive cases recorded in Asia and the Pacific Islands. Consequently a variety of immunologic methods have been used in attempts to improve and simplify the diagnosis of this disease. Practically all of the diagnostic testing, however, has been with animal models and human testing, generally, only with presumptive, rather than confirmed cases. The immunodiagnosis of angiostrongyliasis have recently been reviewed (Cross, 1975), with the conclusion that although various immunologic methods show promise, none appear to have the specificity and sensitivity required for a definitive diagnosis of human disease. In most serological tests antigens are prepared from adult *A. cantonensis* and in the case of the indirect hemagglutination (IHA) test it has been shown that IHA antibody is associated with maturation and fecundity of the parasite. In humans the parasite does not usually reach maturity and it therefore seems unlikely that antibodies associated with fecundity would be readily demonstrable in human

sera. Antigens prepared from third- and fourth-stage larvae would seem to be more appropriate in testing for human infections. Further effort should be directed toward more sensitive methods of detecting antibodies in spinal fluid as reported by Tungkanak *et al.*, (1972) who suggests that antibodies may be synthesized within the CNS.

Scientists have been searching for a method to identify antibodies and antigens in sera and spinal fluid from animals and humans with angiostrongylosis for a number of years. The results for the most part have been discouraging. If a particular test worked in animals it rarely worked in humans. This was particularly true for the IHA, passive cutaneous anaphylaxis (PCA), the complement fixation (CF) and indirect fluorescent antibody (IFA) tests with sera, and the CF, gel-diffusion (G-D) and counterimmunoelectrophoresis (CIE) tests for antibodies and antigens in spinal fluid. For example, in the 3 U.S. Marines only one sera and one spinal fluid was positive by gel-diffusion and CIE while sera from the other two were negative.

A few years ago workers in Europe developed the enzyme linked immunosorbent assay or ELISA test (Engvall and Perlmann, 1972). This assay have been recently used to detect antibodies associated with amoebiasis, schistosomiasis, filariasis and intestinal capillariasis (Cross and Chi, 1977) and since the results were encouraging, the technique was used on sera from monkeys experimentally infected with *A. cantonensis*.

Four monkeys were given a large number of *A. cantonensis* infective-stage larvae by stomach tube and the sera collected before and after infection. The sera were tested for antibody by ELISA using antigens prepared from fourth-stage larvae recovered from the brains of experimentally infected rats. The ELISA values (Table 4) prior to infection in the monkeys ranged from 0.8 - 2.0 and 7 to 18 days after infection from 4.6 - 11.2, indicating rises in antibody titers.

Table 4

ELISA values of sera from monkeys before and after infection with *Angiostrongylus cantonensis**.

Monkey No.	E400/100 min.	
	Pre-infection	Post infection
106	2.0	5.5 (day 7)
111	0.8	11.2 (day 18)
113	1.2	8.3 (day 15)
115	0.9	4.6 (day 15)

* Antigens prepared from fourth-stage larvae of *A. cantonensis*.

Table 5

Sera from U. S. Marines with eosinophilic meningitis tested by ELISA for antibodies to *Angiostrongylus cantonensis*.

Patient No.	E400/100 min.
1	6.6
2	13.2
3*	33.2
Control	2.5

* Serum also positive by G-D and CIE.

Since these results looked promising the sera from the 3 U.S. Marines were tested and ELISA values of 6.6, 13.5 and 33.2 (Table 5) were obtained. The lowest value was obtain for the sera from the sickest Marine and the highest from the one with the least amount of illness, who was also G-D and CIE positive.

The ELISA test used was based upon that of Engvall and Perlmann (1972). In our testing 1 ml of antigen containing 5 μg of protein-ml was used. The antigen was placed into polystyrene tubes and permitted to absorb onto the surface of the tubes overnight at room temperature. The tubes were washed

with borate buffered saline 3 times and 1 ml of test serum diluted 1:1000 added. The tubes were incubated for 6 hours, the serum removed, the tubes washed and alkaline phosphatase, or enzyme labelled anti-human IgG conjugate, added. The tubes were incubated overnight at room temperature, washed, and enzyme substrate containing P-nitrophenyl phosphate added. A yellow color developed and the enzyme reaction was stopped by adding NaOH after 100 minutes.

The color change is proportional to the amount of enzyme conjugate fixed or the amount of antibody captured. The results were read in a spectrophotometer and the ELISA value expressed as the rate of enzyme activity for 100 minutes at an extinction value or wave length of 400 nm. (E400/100 min).

The antigen was a crude preparation of fourth-stage *A. cantonensis* larvae from rat brains. The larvae were ground in a tissue grinder, sonicated, centrifuged and the supernatant used as antigen.

When sera was obtained from the first confirmed case of angiostrongyliasis it was tested by ELISA and found to give a significant reading. The sera was next tested against antigens used in other ELISA tests; *Entamoeba histolytica*, *Toxoplasma gondii*, *Schistosoma japonicum*, *Dirofilaria immitis* and *A. cantonensis*, as well as known positive and negative control sera. Table 6 lists the results and shows the ELISA value for the sera from the confirmed case to be higher for the *A. cantonensis* antigen than for the other antigens.

Sera from people that were positive for other parasitoses were also tested using *A. cantonensis* antigen and the results compared with sera from our confirmed case (Table 7). Although the ELISA values for other helminthiasis were slightly elevated, the readings were significantly higher for angiostrongyliasis than for the other diseases.

The sera collected from the 6 cases of eosinophilic meningitis seen during past few months have all been tested by ELISA. Convalescent sera were obtained from patients from whom worms were recovered and all of the sera were tested simultaneously and the results presented in Table 8. Unfortunately the sera were obtained at different times after onset of symptoms. A serum was considered positive if the ELISA value was twice that of normal control sera. All first sera were considered positive and the ELISA

Table 6

Sera from a confirmed case of angiostrongyliasis tested against other parasitic antigens by ELISA.

Antigens	E400/100 min.		
	A. cantonensis positive	Controls	
		Positive*	Negative
Entamoeba histolytica	0.9	4.1	0.4
Toxoplasma gondii	1.5	7.2	1.3
Schistosoma japonicum	1.5	4.2	0.2
Dirofilaria immitis	3.6	7.2	0
Angiostrongylus cantonensis	11.4	-	0.5

* Human sera positive for respective antigen.

Table 7

ELISA testing of sera from persons with other parasitic diseases against antigens from *Angiostrongylus cantonensis**.

Disease	E400/100 min.	
	Range	Average
Hepatic amoebiasis	0.0 - 1.7	0.3
Intestinal capillariasis	2.0 - 4.1	2.8
Bancroftian filariasis	1.4 - 4.8	3.3
Schistosomiasis	2.9 - 6.3	4.1
Angiostrongyliasis	11.1 - 14.8	12.9
Control	0.7 - 2.4	1.5

* 3-5 sera from each group tested.

Table 8

Sera from six patients with eosinophilic meningitis on Taiwan tested for antibodies to *Angiostrongylus cantonensis* by ELISA.

Patient No.	Serum No.	Days serum obtained after onset	ELISA value E400/100 min.
1*	1	7	13.1
	2	14	41.0
	3	28	23.8
2*	1	21	33.7
	2	29	54.0
3*	1	21	30.8
	2	30	71.7
4	1	28	22.1
5	1	28	28.4
6	1	59	17.1
Normal control	-	-	3.5
Normal control	-	-	4.2

* Worms recovered from spinal fluid.

values for second sera indicate rises in antibody titer. Although the third sera from the first patient showed a decrease in the ELISA value compared to the second serum it was still considered positive. The ELISA values were lower for sera from the presumptive cases, but all were considered positive.

At the present time, the ELISA test looks very promising for the detection of antibodies to *A. cantonensis* but futher testing and refinement should be done using a more purified antigen.

In the meantime, although serologic and biochemical approaches may offer supporting

evidence in the diagnosis of angiostrongylid-induced eosinophilic meningitis, the methods presently available still do not offer conclusive evidence of infection. Until more sensitive and specific tests are available the diagnosis will remain presumptive, based upon clinical manifestations and a history of eating or having contact with animal sources of the infection. The only confirmed diagnosis remains in finding the parasite.

SUMMARY

Angiostrongylus-induced eosinophilic meningitis on Taiwan has been reviewed with emphasis on the clinical aspects and diagnosis of recent cases. In 3 of 6 cases in children, 1½ to 2 years of age, *Angiostrongylus cantonensis* larvae were recovered from spinal fluid. The patients had typical signs and symptoms of angiostrongyliasis with fever, vomiting, stiff neck, headache, eosinophilia and eosinophilic pleocytosis. Three presumptive cases in U. S. Marines who ate *Achatina fulica* on Okinawa were also reported. The young men had pruritis, lower extremity weakness, muscle pain, flaccid paralysis, stiff neck, eosinophila and eosinophilic pleocystosis.

Sera from these cases were examined by the enzyme linked immunosorbent assay or the ELISA test and the results were impressive. In all parasitologically confirmed and presumptive cases the ELISA determination were significantly higher than for the control sera. Paired sera were obtained from the confirmed cases and all showed significant rises in ELISA values or antibody titers. Although the ELISA method shows promise additional testing and refinement of the test is needed.

The diagnosis of angiostrongylid-induced eosinophilic meningitis is considered presumptive unless *A. cantonensis* is demonstrated. A recent history of exposure or eating known intermediate or paratenic hosts is of value but not confirmatory. The definitive diagnosis remains with finding the parasite.

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