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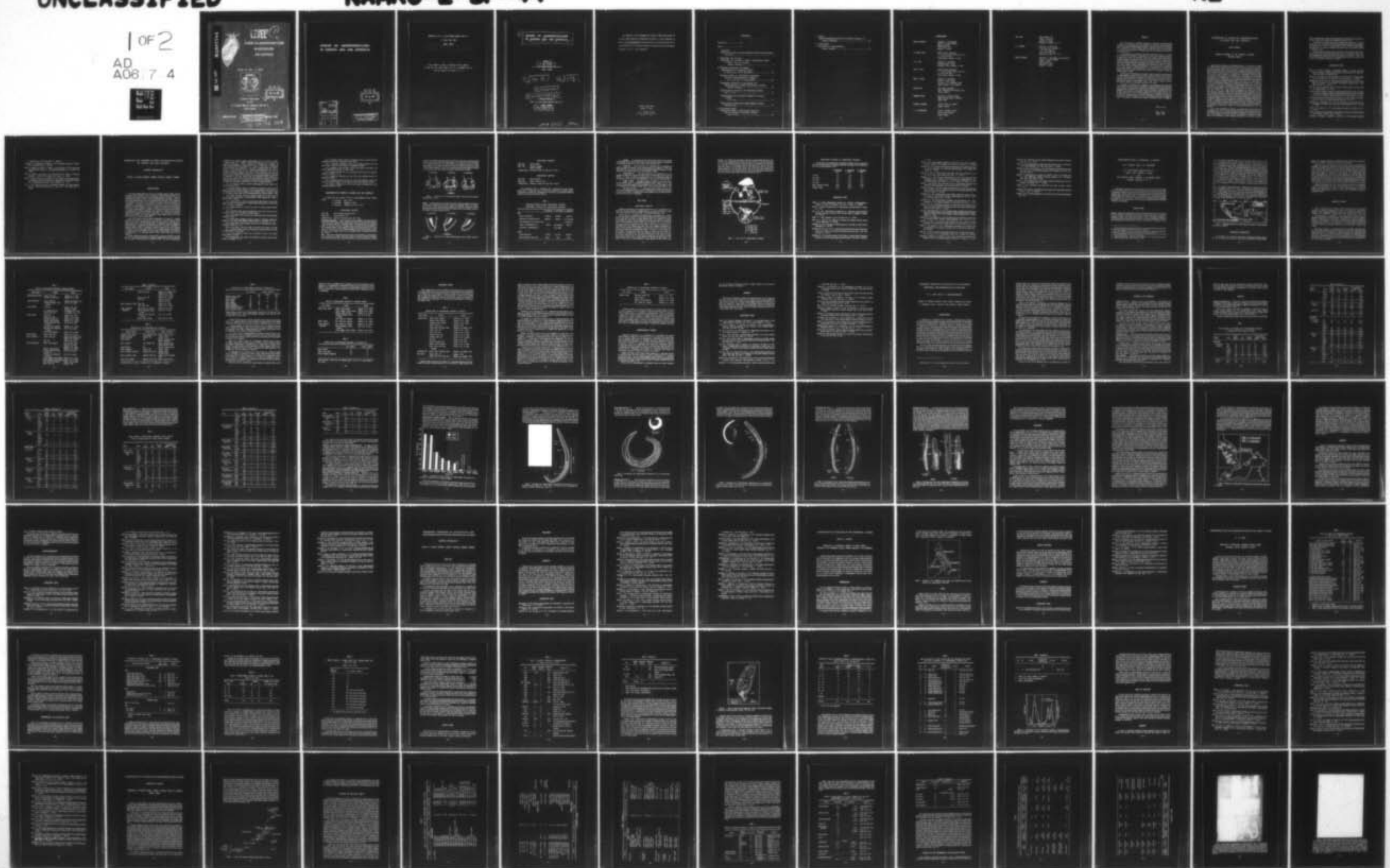

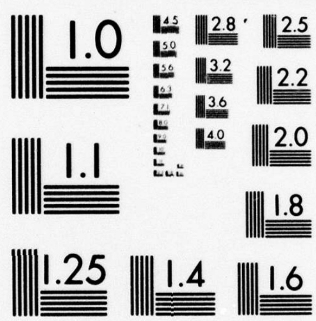

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STUDIES ON ANGIOSTRONGYLIASIS IN EASTERN ASIA AND AUSTRALIA

EDITED BY JOHN H. CROSS

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PREFACE

The primary cause of eosinophilic meningitis or eosinophilic meningoencephalitis in humans in the Asia-Pacific area is the invasion of the central nervous system by larval stages of the nematode *Angiostrongylus cantonensis.* This species along with *A. mackerrasae* and *A. malaysiensis* are found in Eastern Asia and Australia, and all are natural parasites in the lungs of rats. Snails and other mollucan groups serve as intermediate hosts and sources for human infection.

A great deal has been published on the parasite and disease since Dr. Leon Rosen and his associates incriminated *A. cantonensis* as a cause of the eosinophilic meningitis. In recent years, however, new information has become available through studies done by biomedical scientist from endemic areas in Eastern Asia. New species of *Angiostrongylus* have been described and differences in strains of the parasite determined. New intermediate, definitive and paratenic hosts have been identified and consequently new data on transmission have become available. Detailed morphological features have been described and important data on host ecology and geographic distribution of the parasite and the epidemiology of the disease reported. Critical information on the clinical aspects of the disease and diagnosis have been reported, along with new findings on the pathology, immunology and host-parasite relations.

The purpose of this publication is an attempt to present an update on *Angiostrongylus* and angiostrongyliasis in Eastern Asia and Australia. Older information has been reviewed and newer findings presented and discussed. The papers have not been written with any particular group in mind and it is hoped that the material presented will be of value to parasitologists, physicians, epidemiologists, public health workers, ecologists, educators and other biomedical and paramedical scientists in the region.

In addition to the authors who contributed to this monograph, deep appreciation is also extended to Ms. Marisa Chao of the NAMRU-2 Publications Office who spent countless hours preparing the manuscripts for publication, proofreading and correcting manuscripts and galleys, and the myriad of other tasks associated with having a monograph of this type published. Many thanks are also extended to Ms. Loretta Chen and Mrs. Jannie K. Lee, Ms. Lily Wang and to Lt. John W. Hall of NAMRU-2 for their invaluable assistance in the preparation of manuscripts and for making arrangements for publication. Furthermore, the publication of this monograph would not have been possible without the encouragement, support and guidance of Captain Kurt Sorensen, Commanding Officer of NAMRU-2.

John H. Cross

Taipei, Taiwan
January 1979

INTRODUCTION TO STUDIES ON ANGIOSTRONGYLIASIS IN EASTERN ASIA AND AUSTRALIA

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Angiostrongylus cantonensis, a metastrongylid nematode of rats, was first recognized as an important cause of human illness about 20 years ago– some 25 years after the parasite itself was discovered (Chen, 1933) in the course of routine parasitologic examination of domestic rats trapped in Canton, China. Despite prior elucidation of its life cycle and unusual path of migration through the central nervous system of vertebrates (Mackerras and Sandars, 1955), it was not until outbreaks of an unusual type of meningitis of unknown etiology were observed on Pacific islands that the significance of *A. cantonensis* as a human pathogen was perceived. The meningitis seen on the islands was characterized by a pleocytosis consisting in large part of eosinophilic leucocytes, and hence this previously unknown epidemic disorder was termed eosinophilic meningitis (Bailey, 1948; Franco *et al.*, 1960; Rosen *et al.*, 1961). Epidemiologic investigations of the outbreaks led to the discovery of *A. cantonensis* in the brain of a fatal case of eosinophilic meningoencephalitis (Rosen *et al.*, 1962) and the incrimination of the parasite on epidemiologic grounds (Rosen *et al.*, 1967) as the causative agent of the Pacific disease. It now is known that human illness produced by *A. cantonensis* is far more common on the Asian mainland (Punyagupta *et al.*, 1970) than on Pacific islands and it is of interest to consider why its importance was first recognized in the latter area. A plausible explanation is that because of the limited flora and fauna of Pacific islands relatively few infectious diseases of man occur there. This contrasts with the Asian mainland where the abundance of infectious diseases and helminths parasitizing man creates difficulties in differential diagnosis and in the detection of previously unrecognized clinical entities. Actually, *A. cantonensis* was first associated with human disease in Taiwan in 1944 (Nomura and Lin, 1945; Beaver and Rosen, 1964). However, the report was published in Japanese in a Taiwanese medical journal of limited circulation in wartime and this description of a single fatal case remained either unknown, or of unappreciated significance, until many years later.

As the clinical manifestations caused by *A. cantonensis* began to be studied in Asia (Punyagupta *et al.*, 1975; Yii, 1976), anomalies were noted in the geographic distribution of the disease in man as compared with the geographic distribution of the parasite in rodents, and in the pathogenicity of various strains of "*A. cantonensis*" for laboratory primates (Cross, 1979). Investigation of these unexpected findings led to the discovery of another species of *Angiostrongylus*, closely related to *cantonensis*, but apparently much less pathogenic for lower primates and, probably, man (Bhaibulaya and Cross, 1971).

It also should be noted that another newly recognized human disease due to a parasite closely related to *A. cantonensis* has recently been discovered in Central America (Morera and Céspedes, 1971). In view of the large number of species included in *Angiostrongylus* and related genera it would be surprising if other species were not eventually recognized as pathogenic for man.

This monograph will bring the reader up to date on the latest findings on angiostrongyliasis in Eastern Asia and Australia. Unfortunately, there is a large lacuna. This concerns the epidemiology and clinical importance of the parasite in the country in which it first was discovered mainland China. In view of recent political developments, it probably will not be too long before this deficiency is remedied.

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MORPHOLOGY AND TAXONOMY OF MAJOR *ANGIOSTRONGYLUS* SPECIES OF EASTERN ASIA AND AUSTRALIA

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NOMENCLATURE

The genus *Angiostrongylus* was erected for *Strongylus vasorum* (Baillet, 1866) by Kamenskii (1905). Dougherty (1946) in describing *A. gubernaculatus,* established 5 new combinations, placing the following in the genus *Angiostrongylus,* in addition to *A. vasorum: Haemostrongylus raillieti* (Travassos, 1927), *Parastrongylus tateronae* (Baylis, 1928), *Rodentocaulus ondatrae* (Schulz *et al.*, 1933), *Pulmonema cantonensis* (Chen, 1935), *Cardionema ten* (Yamaguti, 1941). Schulz (1951) established two genera, namely, *Rattostrongylus* and *Angiocaulus,* and transferred *A. cantonensis* to the former on the basis of having long spicules, and *A. gubernaculatus* to the latter on the basis of the presence of a gubernaculum. Skrjabin *et al.* (1952) recognized *A. vasorum, A. raillieti, A. tateronae* and *A. ten* as belonging in the genus *Angiostrongylus,* but retained *A. cantonensis* in the genus *Rattostrongylus, A. gubernaculatus* in the genus *Angiocaulus,* and transferred *A. ondatrae* back to the genus *Rodentocaulus.* Yamaguti (1961), without mentioning *A. ten,* accepted the classification proposed by Skrjabin *et al.* (1952), and added *A. blarini* (Ogren, 1954), and *A. soricis* (Soltys, 1953), to the genus *Angiostrongylus.*

Ash (1967) proposed that the genus *Angiostrongylus* to consist of *A. vasorum, A. raillieti, A. tateronae, A. cantonensis, A. ten, A. blarini, A. soricis* and added *A. chabaudi* (Biocca, 1957) and *A. michiganensis* (Ash, 1967). He proposed that *A. ondatrae* and *A. gubernaculatus* remain in the genera *Rodentocaulus* and *Angiocaulus*, respectively. Bhaibulaya (1968) described *A. mackerrasae* and followed Ash (1967) in placing *A. cantonensis* in the genus. Alicata (1968a), in describing *A. sandarsae,* suggested in line with Dougherty (1946), that the presence of a gubernaculum was not of generic significance and transferred *A. ondatrae* and *A. gubernaculatus* back to the genus *Angiostrongylus.* The description of a gubernaculum in *A. cantonensis* (Alicata, 1968b) provided further support for this suggestion.

Drozdz (1970), based mainly on the characters of lateral and dorsal rays and the habitat in the definitive hosts, divided 15 species of *Angiostrongylus* reported at that time into two genera, *Angiostrongylus* and *Stefanskostrongylus.* The genus *Angiostrongylus* comprised of two subgenera. Subgenus *Angiostrongylus* was for *A. vasorum, A. raillieti, A. gubernaculatus* and *A. chabaudi.* Subgenus *Parastrongylus* was for *A. tateronae, A. ondatrae, A. cantonensis, A. sciuri, A. mackerrasae, A. sandrasae* and *A. dujardini.* The genus *Stefanskostrongylus* was established for *A. soricis, A. blarini* and *A. michiganensis.* Following Drozdz's new combination, 4 new species of *Angiostrongylus* were described, namely, *A. schmidti* (Kinsella, 1971), *A. malaysiensis* (Bhaibulaya and Cross, 1971), *A. costaricensis* (Morera and Cespedes, 1971), and *A. minutus* (Ohbayashi *et al.*, 1973). On the basis of possessing markedly reduced dorsal ray, short lateral rays and small simple filariform spicules, Chabaud (1972) established a new genus *Morerastrongylus* and transferred *A. costaricensis* to this genus. However, these new combinations created confusion and, moreover, most workers in Asia and Australia preferred calling the nematodes of this genus *Angiostrongylus*, particularly, *A. cantonensis, A. mackerrasae* and *A. malaysiensis.* Therefore, the names of the nematodes described herein are from the original descriptions.

Up to the present, 19 species in the genus *Angiostrongylus* have been reported from various animals as follows:

(1) *A. vasorum,* (Baillet, 1866) in the pulmonary arteries and the right side of the heart of the domestic dog, *Canis familiaris.* It occurs in Europe (Baillet, 1866), South America (Goncalves, 1961) and Australia although the only published record in Australia is based on the finding of larvae in faeces of a dog showing pulmonary symptoms (Roberts, 1940);

(2) *A. raillieti,* (Travassos, 1927) in the pulmonary arteries and the right ventricle of the crab-eating dog, *Canis azarae,* in Brazil; Dougherty (1946) suggested that it might be identical with *A. vasorum;*

(3) *A. tateronae,* (Baylis, 1928) in the Kemp jerboa, *Tatera kempii* in Ibadan, West Africa. It was found in the stomach, but Baylis (1928) believed that the normal habitat was in the lungs or airpassages;

(4) *A. ondatrae,* (Schulz *et al.*, 1933) in the lungs of the muskrat, *Ondatra zibethica,* in U. S. S. R.;

(5) *A. cantonensis,* (Chen, 1935) in the pulmonary arteries and the right side of heart of rodents, mainly in the Indo-Asian and Pacific regions;

(6) *A. ten,* (Yamaguti, 1941) in the heart of the black-footed marten, *Martes melampus melampus,* in Japan;

(7) *A. gubernaculatus,* (Dougherty, 1946) in the heart of the badger, *Taxidea taxus neglecta* and the striped skunk, *Mephitis mephitis holzneri,* in California, U. S. A.;

(8) *A. blarini,* (Ogren, 1954) encysted in the lungs of the short-tailed shrew, *Blarina brevicauda,* in Illinois, U. S. A.;

(9) *A. soricis,* (Soltys, 1953) in the lungs of the shrew, *Sorex minutus,* in Poland;

(10) *A. chabaudi,* (Biocca, 1957) in the pulmonary arteries and heart of the wild cat, *Felis silvestris,* in central Italy;

(11) *A. sciuri,* (Merdivenci, 1964) from the pulmonary arteries of the squirrel, *Sciurus vulgaris,* in Turkey;

(12) *A. michiganensis,* (Ash, 1967) in the bronchioles of the shrew, *Sorex cinereus cinereus,* in Michigan, U. S. A.;

(13) *A. sandarasae,* (Alicata, 1968) in the pulmonary arteries of rodents, *Mastomys natalensis* and *Gerbil tatera,* in Mozambique, East Africa;

(14) *A. mackerrasae,* (Bhaibulaya, 1968) in the pulmonary arteries and the right side of heart of the rat, *Rattus fuscipes,* in Queensland, Australia;

(15) *A. dujardini,* (Drozdz and Doby, 1970) in blood vessels of the lungs of wild rodent, *Clethrionomys glareolus,* in South France;

(16) *A. schmidti,* (Kinsella, 1971) in the pulmonary arteries of the rice rat, *Oryzomys palustris,* in Florida, U. S. A.;

(17) *A. malaysiensis,* (Bhaibulaya and Cross, 1971) in the pulmonary arteries of *R. jalorensis* in Malaysia;

(18) *A. costaricensis,* (Morera and Cespedes, 1971) in the mesenteric arteries of the cotton rat, *Sigmodon hispidus,* in Costa Rica and other Central American countries;

(19) *A. minutus,* (Ohbayashi *et al.,* 1973) in the lungs of the Japanese shrew mole, *Urotrichus talpoides,* in Japan.

ANGIOSTRONGYLUS SPECIES IN EASTERN ASIA AND AUSTRALIA

In Eastern Asia and Australia 3 species of *Angiostrongylus* have been recorded, namely,

A. cantonensis (Chen, 1935)
A. mackerrasae (Bhaibulaya, 1968)
A. malaysiensis (Bhaibulaya and Cross, 1971).

Angiostrongylus cantonensis

Type host: *Rattus norvegicus* and *Rattus rattus*

Other host: Various species of rats

Location in host: Pulmonary arteries and right side of heart

Morphological description: Body is filariform and tapers slightly at both ends. Epidermis is smooth and bears transverse striae; transparent when alive. The head is simple possessing 3 lips; one dorsal lip which has 2 submedian papillae; 2 subventral lips, each has 1 submedian papilla. At the base of the lips are 8 papillae which are grouped in pairs corresponding to the 4 submedian angles. Cervical papillae are present; each situates laterally on the dorsal surface of the body close to the oesophago-intestinal junction. Buccal capsule is absent; the mouth is seen opening directly into the oesophagus, which is widened before joining the intestine.

Male: Caudal bursa is well-developed, kidney-shaped, single lobed (Fig. 1). The arrangement of the bursal rays is as follows: ventral ray branched at a point two-thirds of its length into a small ventro-ventral and a large latero-ventral ray. Lateral rays arise from a common trunk, the antero-lateral ray is thicker than the others and projected like a thumb, the medio-lateral ray and postero-lateral ray usually originate as a common trunk, the postero-lateral ray is normally shorter than the medio-lateral ray and sometimes reduced to a stump. Externo-dorsal ray is simple and arises from between the lateral and dorsal rays. Dorsal ray is variable, emerging as a short trunk, terminating in several small digitations. Spicules are equal, slender and with conspicuous striations. Gubernaculum is present.

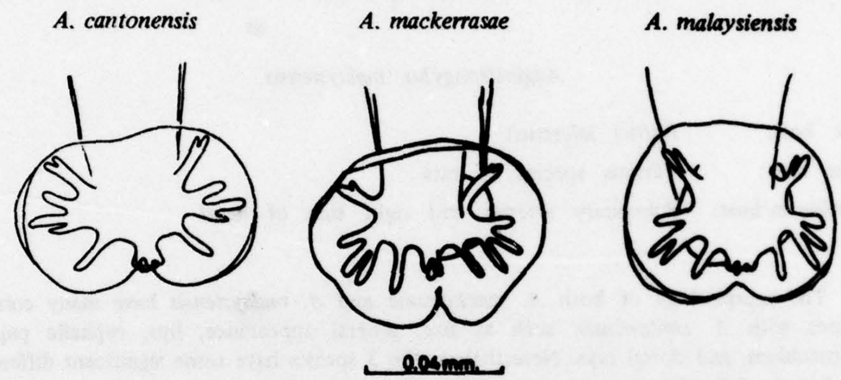

Figure 1. Posterior end of *Angiostrongylus* species of Eastern Asia and Australia showing bursae and rays.

Female: In living specimens, the milky white uterine tubules are spirally wound around the blood-filled intestine and can be seen through the transparent cuticle as a "barber's pole" pattern. A single thin-walled vagina commences at the junction of the uterine tubules, extends posteriorly and opens at the vulva. The posterior end of the female at the tip of the tail has no minute projection (Fig. 2).

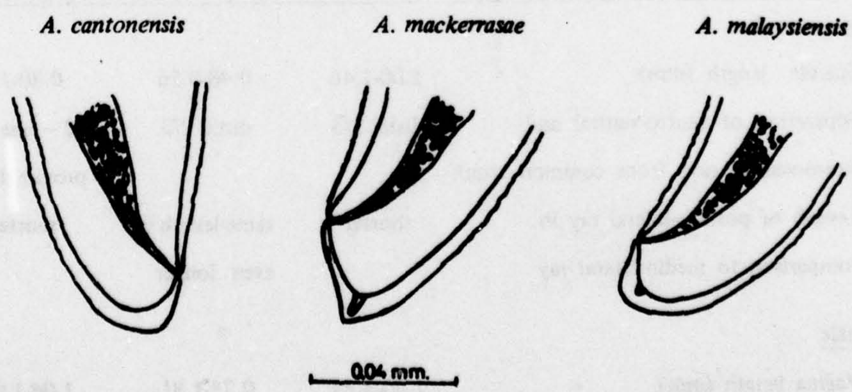

Figure 2. Posterior end of female *Angiostrongylus* species of Eastern Asia and Australia.

Angiostrongylus mackerrasae

Type host: *Rattus fuscipes*

Other host: *Melomys cervinipes*
Rattus norvegicus

Location in host: Pulmonary arteries and right side of heart

Angiostrongylus malaysiensis

Type host: *Rattus jalorensis*

Other host: Various species of rats

Location in host: Pulmonary arteries and right side of heart

The morphology of both *A. mackerrasae* and *A. malaysiensis* have many common features with *A. cantonensis,* such as size, general appearance, lips, cephalic papillae, gubernachlum, and dorsal rays. Nevertheless, the 3 species have some significant differences regarding the following characteristics (Table 1):

Table 1

Morphologic differences among *Angiostrongylus cantonensis,* *Angiostrongylus mackerrasae* and *Angiostrongylus malaysiensis*

	A. cantonensis	*A. mackerrasae*	*A. malaysiensis*
Male			
Spicule: length (mm)	1.00-1.46	0.40-0.56	0.80-1.20
Separation of ventro-ventral and latero-ventral rays from common trunk	distal 2/3	distal 2/3	1/2 – less than proximal 2/3
Length of postero-lateral ray in comparison to medio-lateral ray	shorter	same length or even longer	shorter
Female			
Vagina length (mm)	1.50-3.25	0.75-1.81	1.08-1.94
Minute projection at tip of tail	absent	long	short

1. **Spicules:** *A. cantonensis* has the longest spicule (1.00-1.46 mm), whereas *A. mackerrasce* has the shortest spicule (0.46-0.56 mm). The spicule length of *A. malaysiensis* is intermediate between those of the previous species (0.80-1.20 mm).

2. **Ventral ray:** The ventral rays of *A. cantonensis* and *A. mackerrasae* are similar; the small ventro-ventral ray and large latero-ventral ray originate at a point about distal two-thirds of the common trunk, in *A. malaysiensis* the ventral ray branches at a range from half of the common trunk to less than proximal two-thirds.

3. **Postero-lateral ray:** The postero-lateral rays of *A. cantonensis* and *A. malaysiensis* are similar; they normally are shorter than the mediolateral ray and sometimes reduced to a stump. In contrary the posterolateral ray of *A. mackerrasae* usually has approximately the same length as the medio-lateral ray and sometimes even longer (Fig. 1).

4. **Length of vagina:** The length of vagina correspondes to that of the spicule of the respective species. *A. cantonensis* possesses the longest vagina (1.50-3.25 mm), whereas *A. mackerrasae* possesses the shortest vagina (0.75-1.81 mm). The length of vagina of *A. malaysiensis* is intermediate between those of the previous species (1.08-1.95 mm).

5. **The female tail** (Fig. 2): *A. cantonensis* does not possess any protuberances at the tip of the female tail, whereas female *A. mackerrasae* have a long minute projection at the tip of tail. Female *A. malaysiensis* has a minute projection at the tip of tail but shorter and stouter than that of *A. mackerrasae.*

LIFE CYCLE

Angiostrongylus cantonensis

Mackerras and Sandars (1955) elucidated the life cycle of "*A. cantonensis*" in the laboratory in Brisbane, Queensland, Australia. However, it was later shown that the species used in their experiments were most likely *A. mackerrasae* (Bhaibulaya, 1975).

The life cycle of *A. cantonensis* is an indirect type; an intermediate host is required for some larval stages to develop. The adult males and females live in the pulmonary arteries and the right side of the heart of rodent definitive hosts. After copulation the females lay eggs which are lodged in the small capillaries of the lungs and develop into the first-stage larvae. The first-stage larvae migrate directly from the respiratory tract to the alimentary tract and pass from the definitive host in the faeces. Various species of molluscs serve as intermediate hosts. The first-stage larvae gain access to the molluscan intermediate host either by direct penetration or by ingestion. The larvae moult twice in the muscular tissue and become the second-and third-stage larvae between 7 to 9 and 12 to 16 days, respectively, after infection. Third-stage larvae retain the sheaths of the first and second stages and are infective to the definitive host. The rodent definitive hosts acquire infection through the oral route, the larvae enter the circulation and the majority of the infective third-stage larvae reach the brain in one or two days. Within the brain tissue the larvae migrate to the olfactory lobes and the cerebral hemispheres. During migration the third moult occurs between 4 to 6 days and the fourth-stage larvae leave the sheath behind. The final site in the brain is the subarachnoid space where the larvae moult for the fourth time between 7 to 9 days after infection and become fifth-stage larvae or young adults. The young adults leave the sheath in the subarachnoid space and approximately 10 days after the fourth moult they migrate to the pulmonary arteries. The young adults reach the pulmonary arteries between 26 to 29 days after infection. Most of the worms reach maturity in the pulmonary arteries, but some males are found to reach maturity while still in the subarachnoid space. The prepatent period ranges 42-45 days postinfection (Fig. 3).

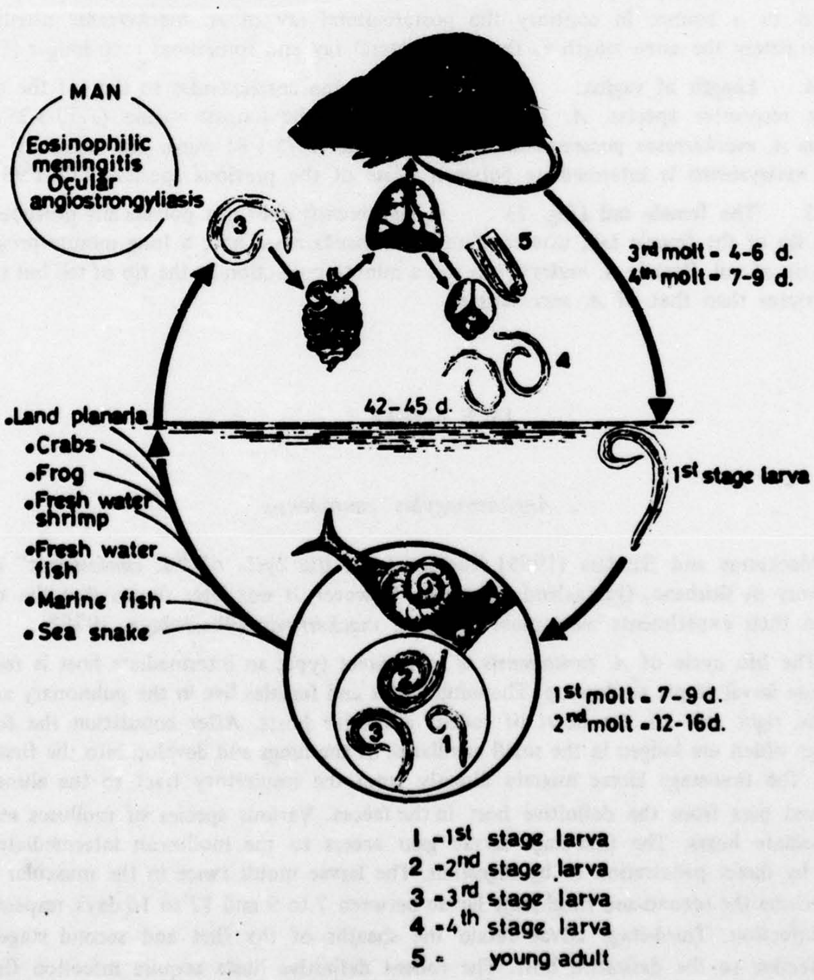

Figure 3. Life cycle of *Angiostrongylus cantonensis.*

Angiostrongylus mackerrasae and *Angiostrongylus malaysiensis*

The life cycles of *A. mackerrasae* and *A. malaysiensis* are similar to that of *A. cantonensis* and the following indicates the comparable significant developmental events (Bhaibulaya, 1975; Lim and Ramachandran, 1979):

	A. cantonensis (day)	*A. mackerrasae* (day)	*A. malaysiensis* (day)
1st moult	7-9	7-10	5-7
2nd moult	12-16	12-16	9-12
3rd moult	4-6	6-10	4-6
4th moult	7-9	10-11	8-12
Reach pulmonary arteries	26-29	25-26	24-28
Prepatent period	42-45	40-42	32

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ANGIOSTRONGYLIASIS IN INDONESIA: A REVIEW*

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Angiostrongylus cantonensis was first incriminated as a cause of eosinophilic meningitis of man in Asia by Nomura and Lin (1945). Approximately 10 years later human cases of eosinophilic meningitis with suspected etiology being *A. cantonensis* were recorded from Indonesia (Smit, 1962 and 1963). Since then parasitologists have sporadically and focally examined rodents and mollusks through the archipelago in search of larval and adult stages of rat lungworms. The current state of knowledge concerning angiostrongyliasis in Indonesia summarized in this review indicates that this zoonosis is widespread and well established throughout the archipelago.

HUMAN CASES

Sumatra. Human cases of eosinophilic meningitis with suspected etiology being *A. cantonensis* were first recorded from Kisaran on the east coast of North Sumatra (Fig. 1) during 1954 through 1957 (Smit, 1962; 1963). Smit described 8 cases of eosinophilic meningitis in Indonesian patients and provided clinical details on 5. All showed eosinophilia of the blood and cerebrospinal fluid (CSF). Cells in the CSF varied from 293 to 4,244 per cu mm,

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and the percentages of eosinophiles varied from 30 to 90. One patient had a severe clinical course with abdominal pains, shortness of breath, heaviness of the appendages, paresis, mental disturbances and incontenence. Symptoms and signs in other patients were less severe, but headaches, fatigue, vertigo and facial paralysis were recorded. All the patients recovered within 2 to 12 weeks and no residual symptoms were observed. In discussing the possible etiology Smit noted that recorded cases of eosinophilic meningitis have 2 distinct etiologies: (1) sporadic cases caused by a variety of helminthic infections and (2) endemic or epidemic cases caused by *Angiostrongylus* spp. He concluded that the Indonesian cases probably belonged to the second group. Subsequently Kwo and Kwo (1968) found *A. cantonensis* in *Rattus rattus diardii* and *R. tiomanicus jaloriensis* from the Medan area of North Sumatra, thus, corroborating the suspected etiology of human cases reported earlier by Smit (1962 and 1963). However, in view of the more recent recovery of *A. malaysiensis* from *R. r. diardii* in Medan (Stafford, 1976b), the latter lungworm may also have been implicated in the cases of eosinophilic meningitis reported by Smit (1962; 1963).

Java. The first and only confirmed case of angiostrongyliasis in Indonesia was recently reported by Widagdo *et al.* (1977). A male *A. cantonensis* was recovered intact from the anterior chamber of the left eye of a 23-year-old woman from Semarang, Central Java (Fig. 1). A useful degree of eye function was preserved following paracentesis. Widagdo *et al.* (1977) described this case and the recovered worm in detail. The worm was identified as a young adult (fifth-stage), male *A. cantonensis* based on the description of Mackerras and Sandars (1955). The possiblity that the worm was *A. malaysiensis*, which is also present in Semarang, Central Java (Carney *et al.*, 1974), was ruled out.

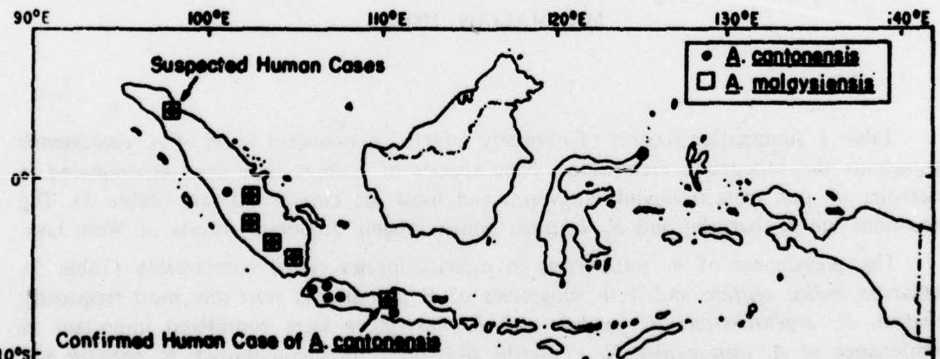

Figure 1. Geographic distribution of *Angiostrongylus cantonensis* and *Angiostrongylus malaysiensis* in Indonesia.

GEOGRAPHIC DISTRIBUTION

The distribution of *A. cantonensis* in the Indonesian archipelago is illustrated in Figure 1. Infections in rodents and/or snails have been found in 9 provinces on 4 major islands:

Sumatra, Java, Sulawesi and Flores. *A. malaysiensis* has been isolated from rodents and/or snails from 4 provinces in Sumatra and from Central Java (Fig. 1).

During the early 1970s thousands of rodents and land snails from throughout the archipelago were examined for appropriate stages of *Angiostrongylus* spp. However, because of the small numbers examined from many localities, negative results may not indicate the true situation. Nevertheless, in some regions of the archipelago sufficient numbers of rodents and/or snails were examined. In Central Sulawesi, for example, nearly 4,000 rodents were examined in conjunction with an intensive study of oriental schistosomiasis but no *Angiostrongylus* spp. were found. In South Sulawesi more than 100 rodents were examined but none was infected with lungworms. Likewise, in East Java and in Bali several hundred snails and 30 rodents have been examined yet *Angiostrongylus* spp. have not been found. In Timor 27 of 55 *Achatina fulica* examined had angiostrongylid-like larvae. However, these larvae did not develop into adult worms in laboratory rats (Carney *et al.*, in press).

Although *Angiostrongylus* spp. have not been reported from Kalimantan, *A. malaysiensis* is endemic in both Sarawak (Lim, 1967; 1970) and Sabah (Lim *et al.*, 1976), Malaysia. Except for the examination of 21 negative *A. fulica* from West Kalimantan (Cross, personal communication) *Angiostrongylus* spp. have not been sought systematically in Kalimantan (Indonesian Borneo).

MAMMALIAN HOSTS

Table 1 summarizes records of naturally infected mammalian hosts of *A. cantonensis* throughout the Indonesian archipelago. Nine species of rodents have been incriminated as reservoirs of this metastrongylid lungworm and most are commensal rats (Table 2). The exceptions are *R. bartelsii* and *R. lepturus* which inhabit mountain forests of West Java.

The prevalences of *A. cantonensis* in rodents species varied considerably (Table 3). *Bandicota indica setifera* and both subspecies of *R. tiomanicus* were the most frequently infected. *R. argentiventer, R. exulans* and *R. norvegicus* were considered important in maintenance of *A. cantonensis. R. r. diardii* and the 2 mountain species, *R. bartelsii* and *R. lepturus,* appeared to be the least involved in this zoonosis. When the same species occurred in both Java and Sumatra infection rates were always higher in Sumatra than in Java.

Data on the intensity of *A. cantonensis* infections in Indonesian rodents are meager with only 3 papers providing quantitative information (Kwo and Kwo, 1968; Margono and Ilahude, 1974; Lim *et al.*, in press). In *R. r. diardii* from Medan, North Sumatra, the mean worm burden was 11 and this relatively high figure accompanied a very high infection rate (55%) in *R. r. diardii* (Kwo and Kwo, 1968). The average infection rate in this rodent species was much lower, 6%. In South Sumatra, Lampung and Jakarta mean worm burdens were low, 3.5, 4.5 and 4, respectively.

Table 1

Records of *Angiostrongylus cantonensis* in Indonesian Rodents

Host species	Locality	Source
Bandicota indica setifera	Jakarta, West Java	Stafford *et al.*, 1976a
	Surakarta, Central Java	Stafford *et al.*, 1976a
Rattus argentiventer	Jakarta, West Java	Margono and Ilahude, 1974
	Baturaja Martapura, South Sumatra	Lim *et al.*, in press
Rattus bartelsii	West Java	Carney *et al.*, 1974
		Wiroreno, in press
	Mt. Masigit, West Java	Van Peenen *et al.*, 1974
	Cibodas, West Java	Van Peenen *et al.*, 1974
Rattus exulans	West Java	Carney, *et al.*, 1974
	Cibuni, West Java	Carney *et al.*, in press
	Semarang, Central Java	Carney *et al.*, in press
	Baturaja, South Sumatra	Carney *et al.*, in press
	Baturaja Martapura, South Sumatra	Lim *et al.*, in press
	Way Abung III, Lampung	Stafford *et al.*, 1976b
	Mulyorejo, Way Abung III, Lampung	Lim *et al.*, in press
	Pagar Dewa, Lampung	Carney *et al.*, in press
Rattus lepturus	Cibodas, West Java	Carney *et al.*, in press
Rattus norvegicus	Jakarta, West Java	Margono and Ilahude, 1974
		Carney *et al.*, in press
	West Java	Carney *et al.*, 1974
Rattus rattus diardii	Medan, North Sumatra	Kwo and Kwo, 1968
		Carney *et al.*, 1974
		Stafford *et al.*, 1976b
	Baturaja, South Sumatra	Stafford *et al.*, 1976b
	Baturaja, Martapura, South Sumatra	Lim *et al.*, in press
	Mulyorejo, Way Abung III, Lampung	Lim *et al.*, in press
	Lubuk Linggau, South Sumatra	Stafford *et al.*, 1976b
	Sungai Tambangan, West Sumatra	Carney *et al.*, in press
	Way Abung III, Lampung	Carney *et al.*, in press
	Bogor, West Java	Wiroreno, 1975

Table 1 (Continued)

Host species	Locality	Source
	Jakarta, West Java	Margono and Ilahude, 1974
		Stafford *et al.*, 1976a
		Carney *et al.*, in press
	Pasuran, West Java	Carney *et al.*, in press
	West Java	Carney *et al.*, 1974
		Wiroreno, in press
Rattus tiomanicus rokuei	West Java	Carney *et al.*, 1974
	Cibuni, West Java	Carney *et al.*, in press
Rattus tiomanicus Jaloriensis	Way Abung III, Lampung	Stafford *et al.*, 1976b
	Baturaja Martapura, South Sumatra	Lim *et al.*, in press
	Mulyorejo, Way Abung III, Lampung	Lim *et al.*, in press
	Medan, North Sumatra	Kwo and Kwo, 1968

Table 2

Mammalian hosts of *Angiostrongylus* spp. in Indonesia

Rodent species	Common name	Habitat Preferences
Bandicota indica setifera	Large Bandicoot Rat	Gardens, Sawah, Scrub
Rattus argentiventer	Ricefield Rat	Gardens, Sawah
Rattus bartelsii	Rat	Primary mountain forest, Sawah occasionally
*Rattus exulans**	Little Pacific Rat	Houses, Gardens, Scrub, Sawah, Secondary forest
Rattus lepturus	Rat	Primary mountain forests
Rattus norvegicus	Norway Rat	Houses, Gardens
*Rattus tiomanicus jaloriensis**	Malaysian Field Rat	Gardens, Scrub, Secondary forest
Rattus tiomanicus rokuei	Malaysian Field Rat	Gardens, Scrub, Secondary forest
*Rattus rattus diardii**	Malaysian House Rat	Houses, Gardens, Scrub

*Confirmed hosts of both *A. cantonensis* and *A. malaysiensis* in Indonesia.

Table 3

Infection rates of *Angiostrongylus cantonensis* in Indonesian rats.

	No. examined	Percent positive*		
		Java	Sumatra	Overall
Bandicota indica setifera	97	44	–	44
Rattus argentiventer	16	21	50	25
Rattus bartelsii	62	3	–	3
Rattus exulans	34	21	25	21
Rattus lepturus	10	10	–	10
Rattus norvegicus	142	15	–	15
Rattus rattus diardii	522	4	12	6
Rattus tiomanicus jaloriensis	54	–	43	43
Rattus tiomanicus rokuei	2	50	–	50

*Figures derived or taken from the following papers: Carney *et al.*, in press; Lim *et al.*, in press; Stafford *et al.*, 1976a, 1976b; Margono and Ilahude, 1974; Wiroreno, 1975, in press; Kwo and Kwo, 1968.

In Medan, North Sumatra Kwo and Kwo (1968) also reported a high mean worm burden (16) in *R. t. jaloriensis*. However, in South Sumatra and Lampung mean worm burdens in this same rodent species were much less, 2.3 and 5.5, respectively (Lim *et al.*, in press).

In Jakarta, Margono and Ilahude (1974) reported that 3 *R. argentiventer* infected with *A. cantonensis* averaged 2 worms per rat and 8 *R. norvegicus* averaged 7 worms per rat. Regrettably, other studies of angiostrongyliasis in Indonesian rodents failed to record any information on the intensity of lungworm infections.

In 2 earlier reports on angiostrongyliasis in Indonesian mammals. (Carney *et al.*, 1974; Stafford *et al.*, 1976a), *R. fulvescens* was listed as a host of *A. cantonensis* in West Java. The mammalian specimen in question has since been re-examined and is now considered *R. lepturus*, not *R. fulvescens*.

Eight of the 9 rodent reservoirs of *A. cantonensis* in Indonesia occur in Java. In metropolitan Jakarta alone 4 rodent species are known to serve as reservoirs, namely, *B. i. setifera*, *R. argentiventer*, *R. norvegicus* and *R. r. diardii*. This is a reflection of the intensity with which rodents have been examined for zoonotic diseases on Java, and, in particular, metropolitan Jakarta, more than an indication that angiostrongyliasis is more established on Java than elsewhere in Indonesia.

A. malaysiensis has been recovered from 3 rodent species: *R. r. diardii*, *R. exulans* and *R. t. jaloriensis* in 3 provinces of Sumatra, namely, North Sumatra, South Sumatra and Lampung (Table 4). Mixed infections of *A. cantonensis* and *A. malaysiensis* occurred in all 3 provinces. *A. malaysiensis* infection rates in rodents are summarized in Table 5. As in the case of *A. cantonensis*, *R. t. jaloriensis* was most often infected and infections were found least often in *R. r. diardii*.

When Kwo and Kwo (1968) reported *Angiostrongylus* in Indonesian rodents, all rat lungworms in Asia were considered to be *A. cantonensis*. In view of the high rates of mixed infections of *A. cantonensis* and *A. malaysiensis* that recently have been reported in Sumatra (Stafford *et al.*, 1976b; Lim *et al.*, in press), the *Angiostrongylus* reported by Kwo and Kwo in the Medan area of North Sumatra may well have been *A. cantonensis* as well as *A. malaysiensis*.

Table 4

Records of *Angiostrongylus malaysiensis* in Indonesian Rodents

Host species	Locality	Source
Rattus rattus diardii	Medan, North Sumatra	Stafford *et al.*, 1976b
	Lubuk, Linggau, South Sumatra	Stafford *et al.*, 1976b
	Baturaja, South Sumatra	Stafford *et al.*, 1976b
	Baturaja Martapura, South Sumatra	Lim *et al.*, in press
	Way Abung III, Lampung	Stafford *et al.*, 1976b
Rattus exulans	Way Abung III, Lampung	Stafford *et al.*, 1976b
Rattus tiomanicus jaloriensis	Way Abung III, Lampung	Stafford *et al.*, 1976b
	Baturaja Martapura, South Sumatra	Lim *et al.*, in press
	Lubuk Linggau, South Sumatra	Stafford *et al.*, 1976b

Table 5

Infection rates of *Angiostrongylus malaysiensis* in Indonesian rats.

	No. examined	Percent positive*
Rattus exulans	13	15
Rattus rattus diardii	174	6
Rattus tiomanicus jaloriensis	34	24

*Figures derived or taken from the following papers: Lim *et al.*, in press; Stafford *et al.*, 1976b.

MOLLUSCAN HOSTS

Three mollusks have so far been incriminated as intermediate hosts of *A. cantonensis* in Indonesia (Table 6). Most of the records are from *A. fulica* simply because this gastropod is an excellent host of *A. cantonensis*, is plentiful throughout most of the islands and is easy to collect and transport alive to a central laboratory. In Jakarta the veronicellid slug, *Laevicaulis alte*, and the large freshwater operculate, *Pila scutata*, were examined, and both implicated in the maintenance of *A. cantonensis* infections (Margono, 1970; Margono and Ilahude, 1974).

Table 6

Molluscan Hosts of *Angiostrongylus cantonensis* in Indonesia

Host species	Locality	Source
Achatina fulica	Jambi, Central Sumatra	Stafford *et al.*, 1976b
	Baturaja, South Sumatra	Stafford *et al.*, 1976b
	Lampung, South Sumatra	Stafford *et al.*, 1976b
	Jakarta, West Java	Margono, 1970
	Jakarta, West Java (larvae only)	Margono and Ilahude, 1974
	Jakarta, West Java	Stafford *et al.*, 1976a
	Ciloto, West Java	Carney *et al.*, in press
	Sukabumi, West Java	Carney *et al.*, in press
	Krawang, West Java	Carney *et al.*, in press
	West Java	Carney *et al.*, 1974
	Semarang, Central Java	Carney *et al.*, 1974
	Menado, North Sulawesi	Carney *et al.*, in press
	Ende, Flores, East Nusa Tenggara	Carney *et al.*, in press
Laevicaulis alte	Jakarta, West Java (larvae only)	Margono and Ilahude, 1974
Pila scutata	Jakarta, West Java	Margono, 1970
	Jakarta, West Java (larvae only)	Margono and Ilahude, 1974

Margono (1970) reported the first isolation of *A. cantonensis* third-stage larvae from mollusks in Indonesia. Angiostrongylid-like larvae were found in *A. fulica* collected from gardens in Jakarta and from *P. scutata* collected from ponds and swamps in the region of Hutan Kapi, Jakarta. In both cases the identification of suspected angiostrongylid larvae was confirmed by feeding these larvae to laboratory-reared rats and recovering and identifying the adult worms. Margono and Ilahude (1974) later examined more than 350 mollusks from the greater Jakarta area and isolated angiostrongylid-like larvae from 35.1% of the *A. fulica*, 35.5% of the *L. alte* and 5.9% of the *P. scutata* specimens they processed. However, no feeding experiments were made to confirm the species of larvae recovered. Wallace and Rosen (1969) considered it impractical to distinguish third-stage larvae of *Angiostrongylus* from those of other metastrongylid nematodes by morphological characteristics alone. Definitive identifications can be accomplished by feeding larvae to laboratory-raised rodents, a procedure that severely limits the number of larvae that can be identified. However, in view of the data Stafford *et al.* (1976a) reported, e.g., 36% of the *A. fulica* in Ancol, Jakarta being infected with third-stage larvae of *A. cantonensis* as determined by feeding experiments, the above infection rates reported by Margono and Ilahude in *A. fulica, L. alte* and *P. scutata* are probably reasonably accurate.

Elsewhere in Java, third-stage larvae of *A. cantonensis* have been isolated from *A. fulica* and confirmed through feeding representative larvae to rodents in Ciloto, Sukabumi and Krawang, West Java (Carney *et al.*, in press) as well as in Semarang, Central Java (Carney *et al.*, 1974). Prevalence rates were not reported.

The finding of *A. cantonensis* third-stage larvae in *A. fulica* from Menado, North Sulawesi and Ende, Flores (Carney *et al.*, in press) considerably extended the known distribution of *A. cantonensis.* Since 25% of the *A. fulica* examined from Menado had *A. cantonensis* larvae, this zoonosis appears to be well established in that northern port city. On the other hand, only 3% of the *A. fulica* examined from Ende, Flores were infected. Angiostrongylid-like larvae also were found in *A. fulica* collected in Kupang, Timor but they did not develop to maturity when fed to laboratory-reared *R. norvegicus, Mus musculus* and *R. exulans* (Carney *et al.*, in press).

Margono and Ilahude (1974) were the only investigators to provide data on the intensity of *A. cantonensis* infections in Indonesian mollusks. In 64 *A. fulica* with larvae, considered *A. cantonensis* on morphological characters, the mean worm burden was 525, ranging from 1 to 6,240, and 22 *L. alte* averaged 299 larvae, ranging from 2 to 2,296 per slug. Ten *P. scutata* had an average of 145, ranging from 1 to 355, larvae per snail. According to Margono and Ilahude (1974) these averages were higher than those reported in the same mollusks in Sarawak, Malaysia (Lim, 1970) and, at least in the case of *A. fulica* and *L. alte,* these infection rates were much lower than in Oahu, Hawaii (Wallace and Rosen, 1969).

A. fulica is the only confirmed molluscan host of *A. malaysiensis* larvae in Indonesia (Table 7). Infections have been found in this species from Semarang, Central Java (Carney *et al.*, 1974) and in 3 provinces of Sumatra, namely, Jambi, South Sumatra and Lampung (Stafford *et al.*, 1976b). In Sumatra, Stafford *et al.* (1976b) reported isolating *A. cantonensis* larvae from *A. fulica* in the Provinces of Jambi, South Sumatra and Lampung. The infection rate was low in Jambi Province (5.5%), but moderately high in both South Sumatran (33.5%) and Lampung (28.6%) Provinces. In all 3 Sumatran provinces mixed infection of *A. cantonensis* and *A. malaysiensis* third-stage larvae have been recovered from *A. fulica.* In Lampung and Jambi 100% of the isolations from infected *A. fulica* were mixed infections.

Table 7

Molluscan hosts of *Angiostrongylus malaysiensis* in Indonesia

Host species	Locality	Source
Achatina fulica	Semarang, Central Java	Carney *et al.*, 1974
	Jambi, Sumatra	Stafford *et al.*, 1976b
	Baturaja, South Sumatra	Stafford *et al.*, 1976b
	Way Abung III, Lampung	Stafford *et al.*, 1976b

Freshwater mollusks are consumed, sometimes raw or partially cooked, by many of the native populations in the outer islands of Indonesia. Pilid snails which were found to harbor third-stage larvae of *A. cantonensis* in Jakarta (Margono, 1970) were confirmed as a major source of human infections in Thailand where most patients with eosinophilic meningitis gave a history of eating raw or partially cooked pilid snails (Punyagupta *et al.*, 1970). As yet, however, the source or sources of human infections in Indonesia remain unknown. Interestingly, however, *A. fulica* in the Indonesian language are known as "Keong racun (ratjun)" or "Poison Snails". The origin of this vernacular name should be researched. Was it due to an illness associated with eating or handling these ubiquitous land snails?

MORPHOLOGICAL STUDIES

Comparative measurements of *A. cantonensis* and *A. malaysiensis* from naturally infected Sumatran rodents were presented by Lim *et al.* (in press). They confirmed that male *A. cantonensis* differed from *A. malaysiensis* in overall length and spicule length, both measurements being larger in *A. cantonensis.* Likewise, the separation of the ventro-lateral and the latero-ventral rays from the main trunk averaged 31.4% in *A. cantonensis* as compared to 54.4% in *A. malaysiensis.* Other meristic and morphological characters of males were similar in both species. Female *A. cantonensis* were longer and wider than *A. malaysiensis;* the vaginal length was also greater in *A. cantonensis.* The projection at the posterior end of *A. malaysiensis* was not present in *A. cantonensis.* Other meristic and morphological characters of females were similar in both species.

Margono and Ilahude (1974) reported meristic characters of *A. cantonensis* recovered from lungs and brain of naturally infected *R. r. diardii, R. argentiventer* and *R. norvegicus* in Jakarta, West Java. They also measured significant meristic characters of *A. cantonensis* larvae from *A. fulica, L. alte* and *P. scutata.* Meristic characters and morphological features of both larvae and adult *A. cantonensis* from Jakarta, Indonesia resembled those given by Mackerras and Sandars (1955).

Other studies of angiostrongyliasis in Indonesian rodents and mollusks regrettably have not documented morphological features or meristic characters of the various angiostrongylid populations encountered.

SUMMARY

Human cases of eosinophilic meningitis with suspected etiology due to rat lungworms have been reported in North Sumatra and one confirmed case of angiostrongyliasis has been reported in Central Java, Indonesia. *Angiostrongylus cantonensis* has been found in rodents and mollusks on 4 major islands in the archipelago: Sumatra, Java, Sulawesi and Flores. Nine rodents and 3 mollusks have been implicated in the maintenance of this zoonosis. *Angiostrongylus malaysiensis* has been found on the islands of Sumatra and Java and in 3 rodent species and 1 land mollusk.

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ECOLOGICAL STUDIES ON *ANGIOSTRONGYLUS MALAYSIENSIS* (NEMATODA: METASTRONGYLIDAE) IN MALAYSIA

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INTRODUCTION

In Malaysia, the rat lungworm known previously as *Angiostrongylus cantonensis* Chen, was first reported in the house rats, *Rattus rattus diardii* (Jent) and *R. exulans* (Peale), by Schacher and Cheong (1960). A preliminary survey of the prevalence of this parasite in house, field and forest rats *(R. r. diardii, R. tiomanicus, R. argentiventer, R. exulans, R. muelleri* and *R. bowersi)* and some molluscan intermediate hosts *(Microparmarion malayanus, Parmarion* sp., and *Lemperula* sp.) from various habitats around Kuala Lumpur was carried out by Lim *et al.* (1962). Later, Lim *et al.* (1965), Lim and Heyneman (1965) and Heyneman and Lim (1965) studied natural infections with this lungworm in field and house rats and various species of intermediate hosts in different habitats in certain parts of Peninsular Malaysia. The parasite was found to be widespread among commensal and forest rats (Lim *et al.*, 1965; Lim and Heyneman, 1965; Lim, 1967) and was found to be transmitted by various land snails and slugs (Lim *et al.*, 1965; Lim and Heyneman, 1965; Lim, 1970; Bisseru and Verghese, 1970). Freshwater snails have also been found to be susceptible intermediate hosts (Lim *et al.*, 1965; Lim and Heyneman, 1965; Lim and Krishnansamy, 1970).

Lim *et al.* (1965) found slight differences in the morphology between the Malaysian taxon of *A. cantonensis* and that originally described by Chen (1935) and by Mackerras and Sandars (1955). Evidence of 'strain' specificity among 3 geographical taxa of *A. cantonensis*, namely the Thai, Hawaiian and Malaysian 'strain' has been observed by Heyneman

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and Lim (1965a; 1966; 1967) and Lim and Heyneman (1968). These studies showed that previously infected rats developed no marked protection against heterologous 'strains', and that there was a high probability that the host was protected against a challenge by the homologous 'strain'. Heyneman and Lim (1966a) and Lim (1970a) in their experimental infection studies of the tree-shrew *(Tupaia glis)*, slow loris *(Nycticebus coucang)* and long-tailed macaque *(Macaca fascicularis)* with the parasite indicate that the slow loris seemed to be entirely refractory to infection, the worm developed in some of the experimental macaques but was not pathogenic to the animals, and some of the tree-shrews were killed by the infection while no worm was found in other tree-shrews. Cross and Fresh (1969) demonstrated that the Malaysian taxon could cause a different pathology in animals from that caused by the Thai and Hawaiian taxa.

Based on these differences in pathogenicity and morphology, Bhaibulaya and Cross (1971) re-studied the adult worms of the Malaysian taxon and found significant morphology differences in both male and female worms with that of *A. cantonensis* from field rats of Formosa, thus they re-described the Malaysian taxon as a new species, *(A. malaysiensis)*. Lim (1975) re-examined *Angiostrongylus* worms previously studied by Lim *et al.* (1965), and agreed with the new description. Subsequently, Lim (1974) also found forest rats naturally infected with *A. malaysiensis* and he postulated that the parasite is indigenous to Malaysia.

The parasite has been reported to be pathogenic to man. The first five human cases of eosinophilic meningoencephalitis due to this parasite in Malaysia were reported from Kuching, Sarawak (Watts, 1969), and the 6th case was found in Kuala Lumpur, Peninsular Malaysia (Bisseru *et al.*, 1972). Larval worms were recovered from 3 of these human cases from Sarawak and Kuala Lumpur. However, the worms recovered in these patients were not available for re-examination and, in both instances, the authors assumed that they were larvae of *A. cantonensis*.

The epidemiology of this disease suggests that direct ingestion of certain food of animal origin was involved in the transmission of the parasite to man. In Thailand, Punyadasni and Punyagupta (1961) and Punyagupta (1965) discovered that patients with eosinophilic meningoencephalitis had ingested pickled snails, *Pila ampullacea*. In the Pacific tropics certain infected freshwater crustaceans, prawns, fish, land crabs, when eaten, transmitted the infective larvae of the parasite to humans (Bailey, 1948; Alicata and Brown, 1962; Rosen *et al.*, 1967; Franco *et al.*, 1960). The giant African snail *(Achatina fulica)*, commonly eaten in certain parts of Taiwan (Formosa), has been implicated in several cases of cerebro-angiostrogyliasis (Hsieh, 1967). Heyneman and Lim (1967a) found that fresh vegetables in Malaysia may also be involved in the transmission of this parasite to man.

The parasite seldom reaches maturity in man who is considered an accidental host. In all known human cases, immature worms have been found only in the cerebrum, cerebellum or spinal cord except in the case of a 5-year old female in South Taiwan who died of the disease, wherein immature worms were recovered from the brain and spinal cord, while mature worms were found in the lungs (Yii *et al.*, 1968). More recently (Sonakul, 1977) reported a degenerate worm in the lung of a Thai female at autopsy. Immature worms have also been recovered from the eye of infected patients (Kanchanaranya and Punyagupta, 1971; Kanchanaranya *et al.*, 1972).

In view of the fact that the Malaysian taxon of the lungworm is a new species, *A.*

malaysiensis, and that the parasite may be pathogenic to man, it was considered particularly appropriate to investigate (1) the prevalence of the parasite in the intermediate and definitive hosts, (2) distributional pattern of the parasite in Malaysia, and (3) the life-cycle of the parasite in laboratory-raised snails and inbred albino Norway rats.

MATERIALS AND METHODS

Collection of hosts. Findings are based on the examination of 16,948 rodents, 1439 insectivores, and 21,016 snails and slugs. Examinations of rodents were made throughout Peninsular Malaysia including Sabah and Sarawak, while that of the molluscs were chiefly made in Peninsular Malaysia, and partly from Sabah and Sarawak. All these hosts were examined from 1962 through 1976.

The molluscs studied include 4 species of land slugs *(Microparmarion malayanus, Laevicaulis alte, Girasia peguensis* and *Lemperula* sp.), 5 species of land snails *(Macrochlamys resplendens, Achatina fulica, Quantula striata, Subulina octona* and *Bradybaena similaris),* and 4 species of aquatic snails *(Pila scutata, Bellamya ingallsiana, Indoplanorbis exustus* and *Lymnaea rubiginosa).* These possible intermediate hosts were all collected from the same localities as those which rodents and insectivores were trapped. The maximum number sampled in any specific habitat was 500; the precise numbers are shown in Table 1.

The rodent hosts examined include 5 species of house and field rats *(R. r. diardii, R. norvegicus, R. exulans, R. tiomanicus* and *R. argentiventer),* a species of insectivore *(Suncus murinus),* 7 species of forest rats *(R. muelleri, R. bowersi, R. surifer, R. annandalei, R. cremoriventer, R. whiteheadi* and *R. sabanus),* and a species of tree-shrew *(Tupaia glis).* All these mammalian hosts were trapped in different ecological habitats; the precise numbers examined are shown in Table 2.

Experimental hosts. Three month-old inbred laboratory albino rats were used for experimental infections with the parasite. Each rat was infected with 300 third or infective stage larvae recovered from naturally infected slugs and snails. The experimental rats were divided into 40 groups of 3 rats each. To study larval development each experimentally infected group of rats was sacrificed daily and the last group killed at 40 days.

Laboratory-raised *L. rubiginosa* fed infected faeces with first-stage larvae collected from experimentally infected rats were used for studies on the development of larval stages. A total of 63 snails were divided into 21 groups of 3 snails each. The first group of experimental snails was sacrificed at 24 hours after ingestion of the infected faeces, the second at 48 hours, and the last sacrificed at 21 days. Larvae recovered from snails sacrificed daily were immersed in 0.85% physiological saline, and preserved in 70% alcohol. The larvae were cleared in lectophenol.

Host examination. Snail bodies and slugs were cut with fine scissors, digested for 4 hours at 37° C in artificial gastric juice, and examined for larvae. Samples of larvae were periodically used to infect white rats to confirm the identification of *A. malaysiensis,* based on morphological examination of the adults.

Rats were brought to the laboratory or field laboratory alive and were killed with chloroform. Brain, spinal cord, heart and lungs were removed and searched for worms. The worms collected were preserved in 5% glycerol in 70% alcohol. The worms were cleared in lactophenol before examination and measurement of the immature, adult and larvae were made by ocular micrometer.

RESULTS

Molluscan intermediate host. Amongst the 4 species of land slugs collected *M. malayanus* is abundant in scrub and oilpalm, while *L. alte* is abundant in town, scrub, lalang and oilpalm; both species are also common in other habitats. *G. peguensis* and *Lemperula* sp. are confined to scrub and fringe vegetation (Table 1).

Three of the 5 species of land snails, *M. resplendens, A. fulica* and *S. octona* are abundant. Ricefields appeared to be a very suitable habitat for all 3 species, although *A. fulica* was present, it was less common. No *S. octona* was collected in rubber estates. *Quantula striata* and *Bradybaena similaris* are less common (Table 1).

Among the habitats sampled, oilpalm seemed to be the best habitat for all land snails.

Table 1

Natural prevalence of third-stage larvae of *Angiostrongylus malaysiensis* in molluscs from various habitats in Malaysia

Species of molluscs	Habitat	No. of molluscs examined	No. positive	Percentage positive	No. of larvae per positive host: Average	Range
Land Slugs						
Microparmarion malayanus	Town	241	14	5.8	142	5-714
	Scrub	500	248	49.6	245	72-1585
	Lalang	145	45	31.0	101	3-892
	Ricefield	175	52	29.7	75	12-362
	Oilpalm	500	365	73.0	298	101-3878
	Rubber	121	75	61.9	121	7-878
	Total	1682	799	47.5	236.6	3-3878
Laevicaulis alte	Town	500	8	1.6	12	2-18
	Scrub	500	25	5.0	84	45-750

Table 1 (Continued 1)

Species of molluscs	Habitat	No. of molluscs examined	No. positive	Percentage positive	No. of larvae per positive host: Average	Range
	Lalang	500	34	6.8	22	2-115
	Ricefield	248	14	5.6	7	4-24
	Oilpalm	500	122	24.4	252	18-2540
	Rubber	251	12	4.8	6	5-28
	Total	2499	215	8.6	157.5	2-2540
Girasia peguensia	Scrub	500	110	22.0	272	75-848
	Fringe	500	127	25.4	268	17-755
	Total	1000	237	23.7	269.9	17-848
Lemperula sp.	Scrub	342	32	9.4	14	4-121
	Fringe	368	11	2.9	8	2-22
	Total	710	43	6.1	12.5	2-121
Land Snails						
Macrochlamys resplendens	Town	500	14	2.8	145	21-872
	Scrub	500	155	31.0	183	12-1542
	Lalang	–	–	–	–	–
	Ricefield	–	–	–	–	–
	Oilpalm	500	352	70.4	285	45-2581
	Rubber	500	29	5.8	61	4-135
	Total	2000	550	27.5	240.9	4-2581
Achatina fulica	Town	500	162	32.4	112	16-1415
	Scrub	500	98	19.6	87	24-784
	Lalang	500	14	2.8	29	6-48
	Ricefield	175	12	1.7	10	3-18
	Oilpalm	500	18	3.6	434	242-4125
	Rubber	500	4	0.8	12	3-16
	Total	2675	308	11.5	113.8	3-4125
Quantula striata	Town	–	–	–	–	–
	Scrub	500	48	9.6	12	4-72
	Lalang	500	0	0	0	0
	Ricefield	–	–	–	–	–
	Oilpalm	500	9	1.8	42	15-95
	Rubber	–	–	–	–	–
	Total	1500	57	3.8	16.7	4-95

Table 1 (Continued 2)

Species of molluscs	Habitat	No. of molluscs examined	No. positive	Percentage positive	No. of larvae per positive host: Average	Range
Subulina octona	Town	500	2	0.4	6	5-7
	Scrub	500	8	1.6	4	2-8
	Lalang	500	0	0	0	0
	Ricefield	–	–	–	–	–
	Oilpalm	500	10	2.0	11	2-21
	Rubber	–	–	–	–	–
	Total	2000	20	1.0	12.8	2-21
Bradybaena similaris	Town	–	–	–	–	–
	Scrub	500	4	0.8	3	1-4
	Lalang	–	–	–	–	–
	Ricefield	–	–	–	–	–
	Oilpalm	500	2	0.4	2	1-3
	Rubber	–	–	–	–	–
	Total	1000	6	0.3	2.7	1-4
Aquatic Snails						
Pila scututu	Ricefield	500	124	24.8	18	5-150
	Pond	500	78	15.6	12	3-87
	Stream	500	17	3.4	6	2-25
	Total	1500	219	14.6	14.9	2-150
Bellamyia ingallsiana	Ricefield	500	84	16.8	16	3-115
	Pond	500	12	2.4	4	2-28
	Stream	500	5	1.0	3	1-11
	Total	1500	101	6.7	13.9	1-115
Indoplanorbis exustus	Ricefield	500	105	21.0	5	3-28
	Pond	500	8	1.6	2	1-9
	Stream	500	3	0.6	2	1-4
	Total	1500	116	7.7	4.7	1-28
Lymnaea rubiginosa	Ricefield	500	52	10.4	3	1-34
	Pond	500	0	0	0	0
	Stream	500	0	0	0	0
	Total	1500	52	10.4	3.0	1-34

Rodent definitive hosts. *R. r. diardii* is the common and most abundant house rat in town. Although this rat is common in other habitats, it is always associated with human habitations. *R. exulans* (Polynesian rat) is fairly abundant in ricefields and in rubber, but less common in other habitats. *R. tiomanicus* (Malaysian wood rat) is most abundant in oilpalm and although common in other habitats, it is strictly a field rat and is not found near human habitations. The density is low. *R. argentiventer* (ricefield rat) is most abundant in ricefields and lalang; the density is low in scrub, oilpalm and rubber estate habitats. Like *R. tiomanicus*, this rat is not found near human habitations. *R. norvegicus* (seaport rat) is a house rat strictly confined to seaport and in towns near seaports, and *Suncus murinus* (house shrew) inhabits residential areas, but also invades fields near human habitation (Table 2).

Table 2

Natural prevalence of *Angiostrongylus malaysiensis* in house, field and forest rats including shrews from various habitats in Malaysia

Species	Habitat	No. examined	No. positive	Percentage positive	No. of worm per positive host: Average	Range
Rattus r. diardii	Town	2712	252	9.3	4	2-18
(Malaysian House	Scrub	388	124	31.9	8	3-28
Rat)	Lalang	140	5	12.5	2	1-4
	Ricefield	304	45	14.8	3	2-12
	Oilpalm	124	60	48.4	6	2-24
	Rubber	240	10	4.2	2	1-12
	Total	3808	496	13.0	5.1	1-28
Rattus exulans	Town	172	8	4.7	2	2-4
(Polynesian Rat)	Scrub	140	34	24.3	4	2-43
	Lalang	192	12	1.6	2	2-10
	Ricefield	344	86	25.0	2	1-8
	Oilpalm	44	20	45.5	4	1-4
	Rubber	336	22	6.5	2	1-6
	Total	1228	182	14.8	2.6	1-43
Rattus tiomanicus	Town	—	—	—	—	—
(Malaysian Wood	Scrub	576	200	34.7	8	6-16
Rat)	Lalang	552	60	10.9	4	2-12

Table 2 (Continued 1)

Species	Habitat	No. examined	No. positive	Percentage positive	No. of worm per positive host Average	Range
	Ricefield	460	115	25.0	5	1-22
	Oilpalm	2945	1031	35.0	10	2-72
	Rubber	272	27	9.9	4	1-8
	Total	4805	1433	29.8	8.9	1-72
Rattus argentiventer (Ricefield Rat)	Town	—	—	—	—	—
	Scrub	136	68	50.0	8	2-46
	Lalang	352	40	11.4	7	1-18
	Ricefield	841	124	14.7	8	2-54
	Oilpalm	180	54	30.0	16	1-24
	Rubber	24	2	8.3	4	1-8
	Total	1533	288	18.8	9.3	1-54
Rattus norvegicus (Seaport Rat)	Town	472	94	19.9	11	4-28
	Seaport	815	203	24.9	12	6-48
	Total	1287	297	23.1	11.7	4-48
Suncus murinus (House Shrew)	Town	545	2	0.4	190	20-360*
	Ricefield	114	0	0	0	0
	Total	659	2	0.3	190	20-360
Rattus muelleri (Muller's Forest Rat)	Fringe	454	7	1.5	4	1-8
	Forest	648	0	0	0	0
	Total	1102	7	0.6	4	1-8
Rattus bowersi (Bower's Forest Rat)	Fringe	48	3	6.3	5	2-8
	Forest	424	0	0	0	0
	Total	472	3	0.6	5	2-8
Rattus surifer (Spiny-furred Rat)	Fringe	185	2	1.1	3	2-4
	Forest	328	0	0	0	0
	Total	413	2	0.5	3	2-4
Rattus annanadalei (Annandale's Rat)	Fringe	295	2	0.7	4	1-3
	Forest	48	0	0	0	0
	Total	343	2	0.6	4	1-3
Rattus cremoriventer (Pencil-tailed Forest Rat)	Fringe	112	5	4.5	7	3-12
	Forest	215	0	0	0	0
	Total	327	5	1.5	7	3-12

Table 2 (Continued 2)

Species	Habitat	No. examined	No. positive	Percentage positive	No. of worm per positive host: Average	Range
Rattus whiteheadi	Fringe	285	1	0.4	3	0-3
(Whitehead's Forest	Forest	348	0	0	0	0
Rat)	Total	633	1	0.2	3	0-3
Rattus sabanus	Fringe	212	2	0.9	4	2-6
(Long-tailed Forest	Forest	785	0	0	0	0
Rat)	Total	997	2	0.2	4	2-6
Tupara glis	Fringe	389	1	0.3	3	0-3
(Tree shrew)	Forest	391	0	0	0	0
	Total	780	1	0.1	3	0-3

All 7 species of forest rats listed in Table 2 are common in primary and secondary forests and they are also found in fringe habitats adjacent to the forest. *T. glis* (tree-shrew) is common both in the fringe and forest habitats.

Prevalence of natural infections in molluscan intermediate hosts. The highest prevalence of natural infection among the 4 species of slugs examined were *M. malayanus* and *G. peguensis* being 47.5% and 23.7%, the lowest being 8.6% for *L. alte* and 6.1% for *Lemperula* sp., respectively. There was no difference in the worm-load between *M. malayanus* and *G. peguensis* with an average of 236.6 and 269.9 per infected slug, while that of *L. alte* and *Lemperula* sp. had averages of 157.5 and 12.5 per infected slug (Table 1).

Of the 5 species of land snails, *M. resplendens* had the highest prevalence of natural infection, 27.5% compared to 11.5% in *A. fulica.* The lowest were observed in *Q. striata, S. octona* and *B. similaris* with rates of 3.8%, 1.0%, 0.3%, respectively. The worm-load of these snails averaged 240.9, 113.9, 16.7, 12.8 and 2.7 per infected snail, respectively.

The highest prevalences of natural infection among the aquatic snails, *P. scutata* and *L. rubiginosa* were found with rates of 14.6% and 10.4%; the lowest were *B. ingallsiana* and *I. exustus* being 6.7% and 7.7%, respectively. There was no marked difference in the worm-load between *P. scutata* and *B. ingallsiana* averaging 14.9 and 13.9 per infected snail, respectively. Similarly, no marked differences in the worm-loads were observed in *I. exustus* and *L. rubiginosa,* averaging 4.7 and 3.0 per infected snail, respectively.

Prevalence of natural infection in definitive rodent hosts. Among the house rats, *R. norvegicus* was observed to have an infection rate or percentage positive of 23.1% compared to 13.0% in *R. r. diardii.* Of the field rats, 29.8% of *R. tiomanicus* was found infected compared to 18.8% in *R. argentiventer* and 14.8% in *R. exulans.* In the house-shrew *(S. murinus)* only 0.3% were positive. No marked difference in the infection rate was observed between the 7 species of forest rats, the rates ranged from 0.2%-1.5%, while in the tree-shrew, *(T. glis),* the infection rate was only 0.1% (Table 2).

R. norvegicus had the highest worm-load averaging 11.3 worms per infected animal compared to 5.1 in *R. r. diardii.* No marked difference was observed between *R. tionamicus* and *R. argentiventer;* the worm-load averaging 8.9 and 9.3 per infected animal. The lowest worm-load was in *R. exulans* with an average of 2.6 worms per infected animal. *R. cremoriventer* had the highest average worm-load of 7 worms per infected animal, followed by 5 in *R. bowersi.* No difference was observed in *R. muelleri, R. surifer, R. annandalei, R. sabanus* and *R. whiteheadi;* the average worm-loads ranged from 3-4 worms per infected animal, while only a single *T. glis* was infected with 3 worms (Table 2).

Infection levels in different habitats. Figure 1 shows the infection levels among the intermediate and definitive hosts in different habitats. Oilpalm hatibats had the highest molluscan infection rate with 25.1%, followed 16.8% in scrub. No marked difference was found between fringe and ricefield habitats; the rate being 15.9% and 14.3%. Similarly, no difference was observed between town and ricefield habitats with rates of 8.9% and 8.7%, respectively. Lower rates of 4.9% and 4.3% were found in ponds and lalangs, while the lowest of 1.3% was observed in stream habitats.

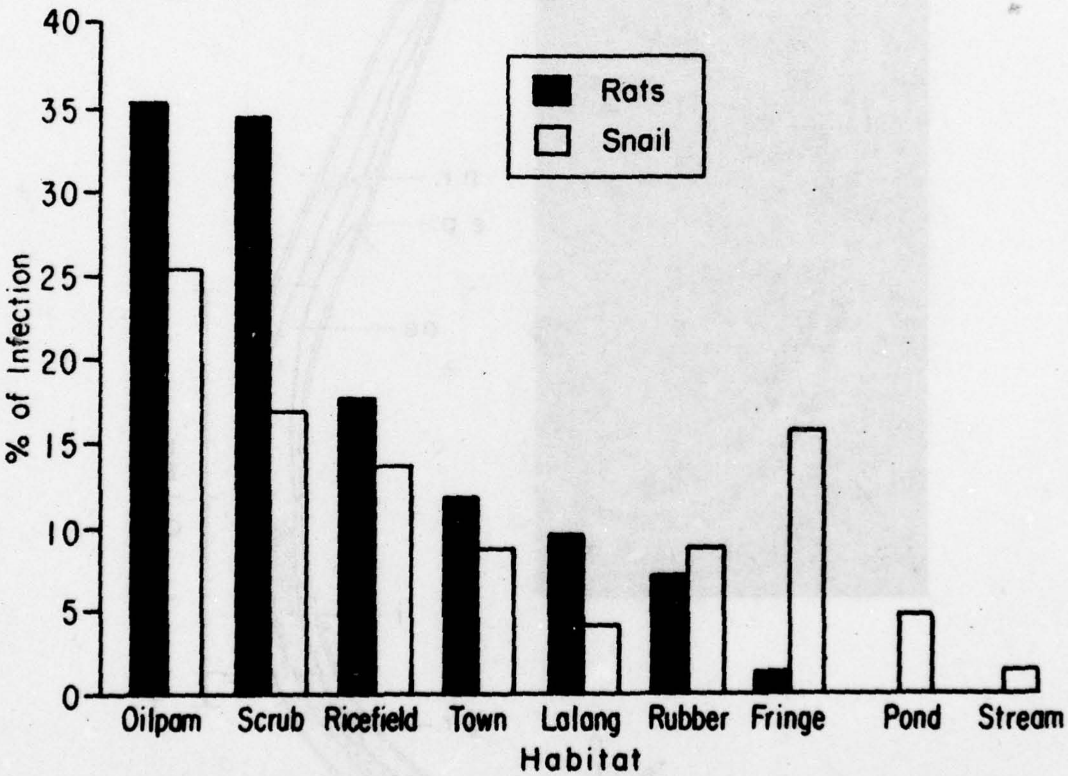

Figure 1. Comparison of natural infections of *Angiostrongylus malaysiensis* in intermediate and difinitive hosts in different habitats.

There were no differences in rodent infection rates between oilpalm and scrub habitats, the rate being 35.4% and 34.4% respectively. Ricefields had a higher infection rate with 17.9%, followed by 11.9% in towns. In lalang habitats the infection rate was 9.5% and 6.9%

in rubber habitats. Fringe habitats had the lowest rate with only 1.2%.

Life-cycle of Angiostrongylus malaysiensis in experimental intermediate and definitive hosts.

First-stage larvae (Fig. 2). First-stage larvae of *A. malaysiensis* recovered from feces of experimentally infected inbred laboratory rats move actively in 0.85% physiological saline. They are slender, with lateral alae extending almost the entire body length. The oesophagus is rhabditoid shaped, and occupies most of the anterior half of the body. The genital rudiment is situated slightly anterior to the mid-intestine. The tail is slightly notched and pointed. The mean length and width (100 specimens) of these larvae were 0.247 ± 0.002 mm. The mean length and width of 100 eggs were 0.06 ± 0.01 and 0.041 ± 0.003 mm respectively.

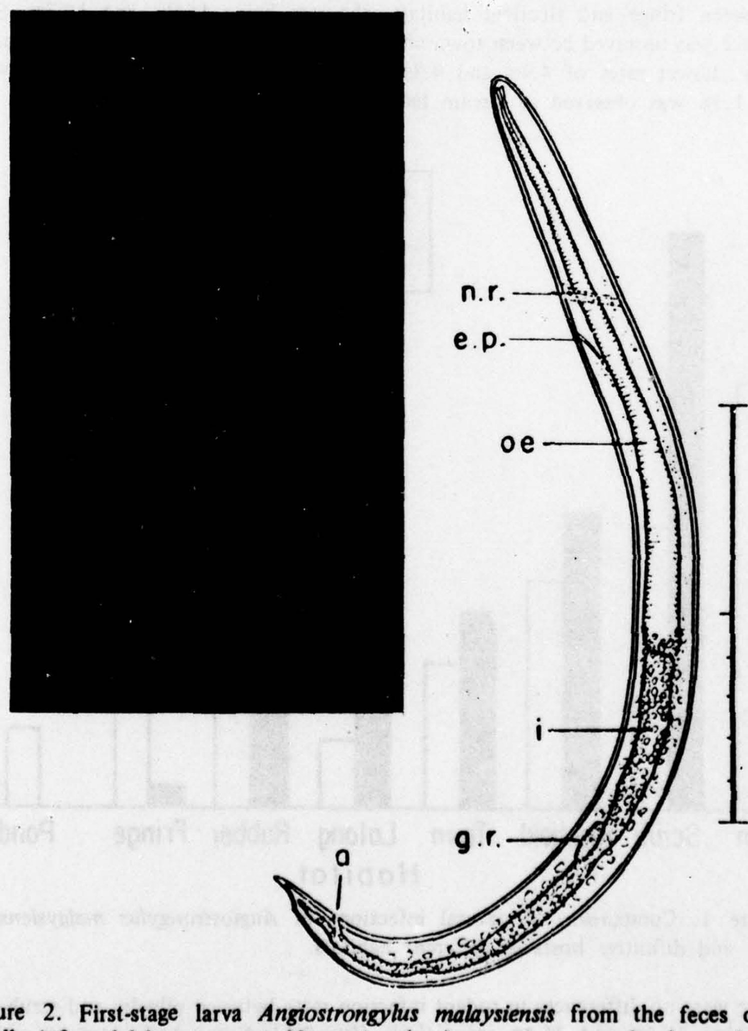

Figure 2. First-stage larva *Angiostrongylus malaysiensis* from the feces of an experimentally infected laboratory rat. Nerve ring (n.r.), excretory port (e.p.), esophagus (oe), intestine (i), genital rudiment (g.r.), anus (a).

Second-stage larvae (Fig. 3). The first molt was observed 5 to 7 days after infection in the snails. The mean length and width (30 specimens) were 0.327 ± 0.064 and 0.027 ± 0.022 mm, respectively. Some of the second-stage larvae retained the sheath of the first-stage. The refractile granules were more intense and larger in size and obscured the entire internal organs in 7 day-old larvae.

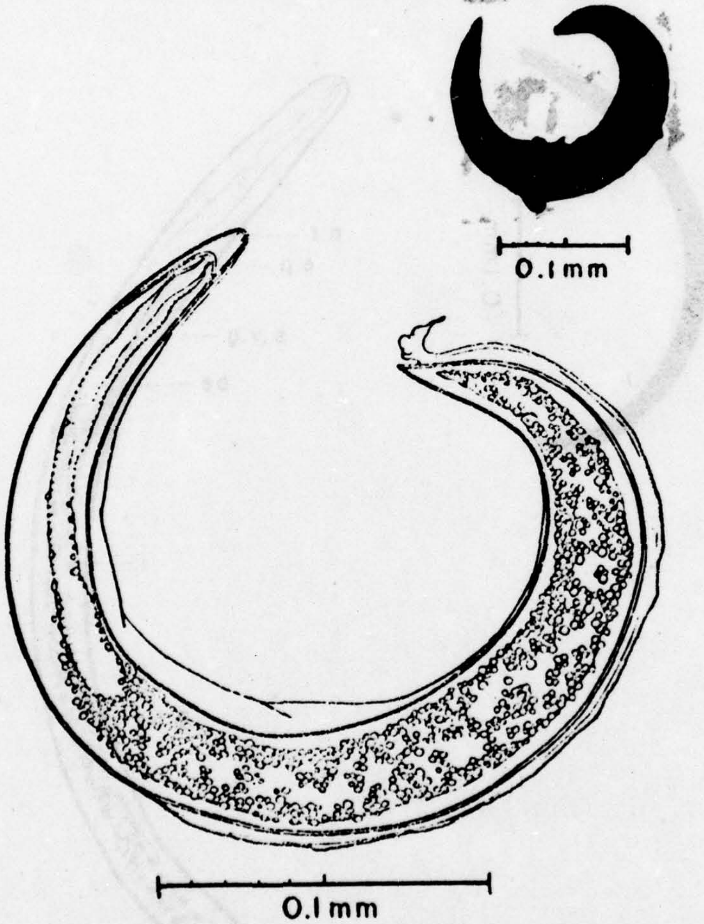

Figure 3. Second-stage larva *Angiostrongylus malaysiensis* from an experimentally infected snail.

Third-stage larvae (Fig. 4). The second moult was observed 9-12 days after infection in snails. Some of the third-stage larvae retained the sheath of the first and second-stages. All larvae recovered at 16 days were in the third-stage (third-stage larvae were found to be infective to rats). The rhabditoid esophagus with cuticular lining, nerve ring, excretory pore and anus were now clearly seen. The genital rudiment was situated at approximately two-thirds of the body length from the posterior end. The intestine tapered sharply from behind the esophagointestinal junction, extending to the anus as a narrow structure between 2 subventral glands. In the body cavity the subventral glands characterized by fine granules, extended slightly posterior from the nerve ring to slightly anterior to the anus. The tail tapered smoothly at this stage to a fine pointed end at 15 days. The mean length and width (30 specimens) of 16 day-old third-stage larvae were 0.50 ± 0.02 and 0.03 ± 0.02 mm, respectively.

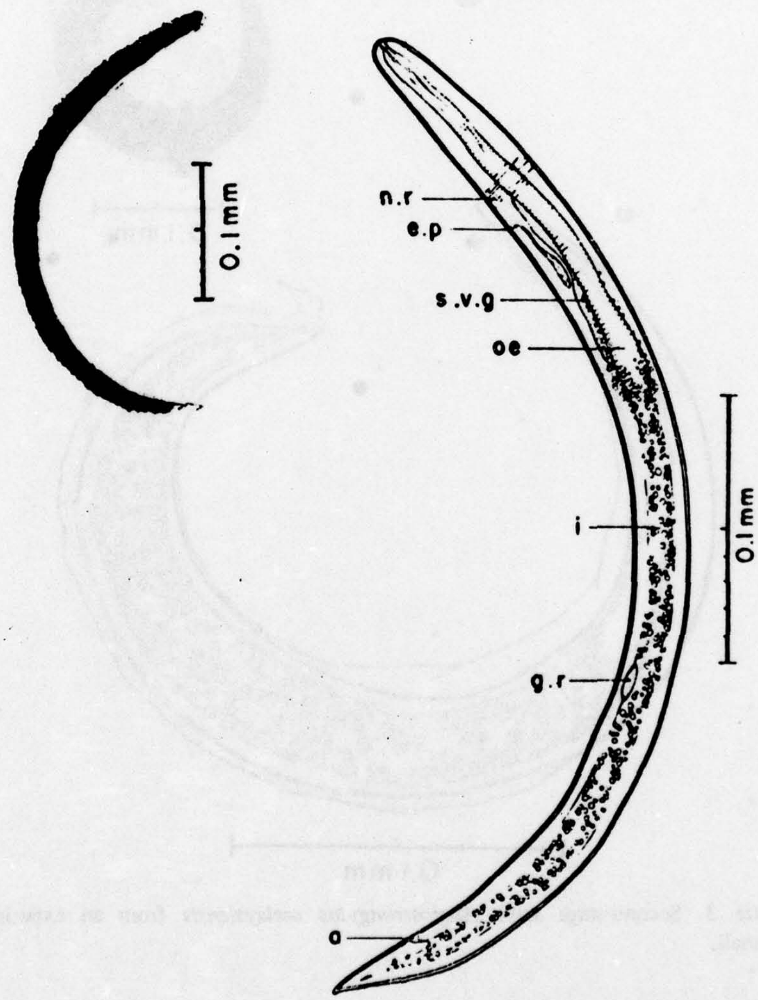

Figure 4. Third-stage larva *Angiostrongylus malaysiensis* from an experimentally infected snail. Nerve ring (n.r.), excretory pore (e.p.), subventral gland (s.v.g.), esophagus (oe), intestine (i), genital rudiment (g.r.), anus (a).

Fourth-stage larvae (Fig. 5). The third moult was observed in the brain of experimentally infected rats 4 to 6 days after infection. The sexes are clearly seen at this stage. The sex of the male larvae could be distinguishable by a bulge at the posterior end and a single reproductive tract which joined the rectum and occupied about the posterior third of the body. The spicule pouch and developing spicules were striated dorsal to the cloaca. In the females the developing reproductive tract extended from the posterior part of the body. The intestine of both males and females were filled with large refractile granules and the subventral glands were short. The mean length and width (30 specimens) of these larvae at 6 days postinfection were 0.77 ± 0.23 and 0.44 ± 0.55 mm, respectively.

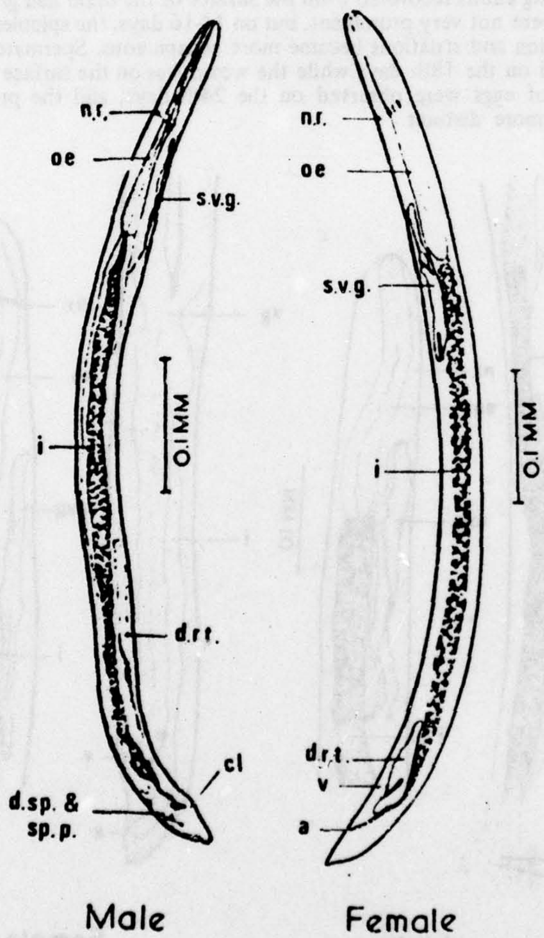

Figure 5. Fourth-stage male and female larvae *Angiostrongylus malaysiensis* from the brain of an experimentally infected rat after 6 days. Nerve ring (n.r.), esophagus (oe), subventral gland (s.v.g.), intestine (i), developing reproductive tract (d.r.t.), vulva (v), cloaca (cl), developing spicule (d.sp.), spicule pouch (sp.p.), anus (a).

Fifth-stage larvae (Fig. 6). The fourth moult was observed in the subarachnoid space of the brain 8-12 days after infection. Young adults left their sheaths and continued developing until they migrated to the lungs. The young adults migrated to the surface of the brain 12 days after infection. The mean length and width of 12 day-old males (30 specimens) were 3.23 ± 0.38 and 0.07 ± 0.01 mm and that of the females were 3.62 ± 0.65 and 0.08 ± 0.01 mm, respectively. The males possessed a small bursa and rays which were similar to those of the adults. The spicule pouch and spicules were present, but no cuticle was deposited in the spicules. The male reproductive tract occupied about the posterior half of the body; the cloaca was completely formed. In the females, the projection at the posterior end can be seen at the 5th day. The reproductive tract occupied the posterior half of the body. On the 13th day of infection young adults recovered from the surface of the brain had grown further. In the males, the spicules were not very prominent, but on 14-16 days, the spicules gradually revealed more cuticle deposition and striations became more conspicuous. Spermatozoa in the seminal vesicle were observed on the 18th days while the worm was on the surface of the brain. In the females, formation of eggs were observed on the 24th days, and the projection at the tip of the tail became more distinct.

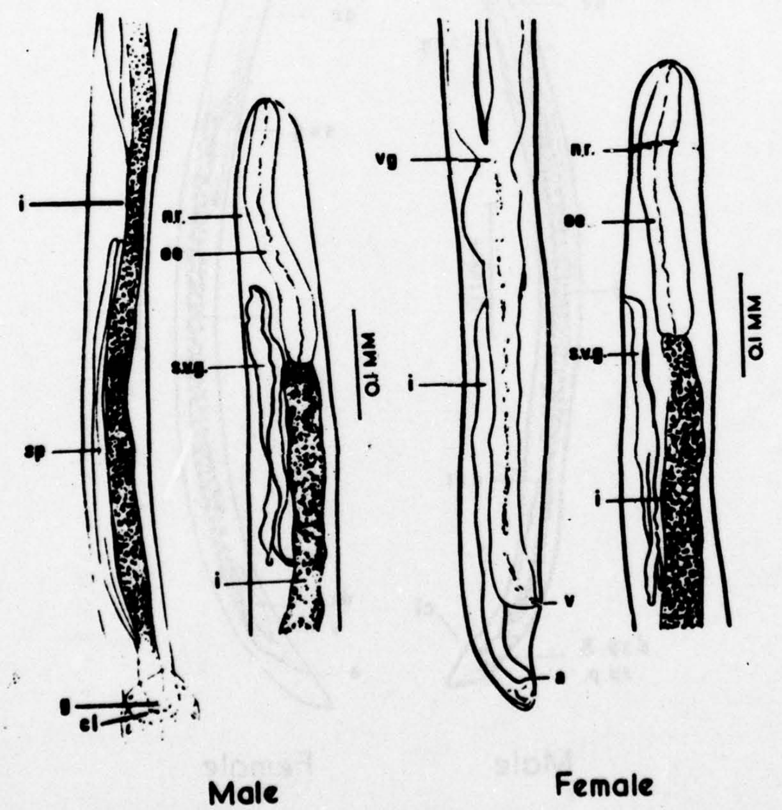

Figure 6. Fifty-stage male and female *Angiostrongylus malaysiensis* from the brain of an experimentally infected rat after 12 days. Nerve ring (n.r.), esophague (oe), subventral gland (s.v.g.), intestine (i), vagina (vg), vulva (v), anus (a), spicule (sp), gubernaculum (g), cloaca (cl).

Most of the worms reached the pulmonary arteries 24 to 28 days after infection. On the 21st day, spermatozoa were found fully developed in the males. In the females, the entire reproductive tract was filled with eggs on the 31st day and larvae were found in the lung tissues of a rat. A few first-stage larvae began to appear in the rat feces on the 32nd day, and on the 35th day numerous larvae were observed in the feces. The mean length and width (30 specimens) of 40 day-old adult males were 17.7 ± 0.60 and 0.26 ± 0.02 mm and that of the females were 21.7 ± 1.02 and 0.35 ± 0.02 mm, respectively.

DISCUSSION

The data available on the intermediate molluscan hosts of *A. malaysiensis* indicate that *M. malayanus* and *L. alte* are 2 of the 4 most abundant land slugs found inhabiting all the habitats studied (town, scrub, lalang, ricefield, oilpalm and rubber), while *G. peguensis* and *Lemperula* sp. are more habitat specific being found only in scrub and fringe habitats. The high density of these land slugs in oilpalm, scrub and fringe habitats was probably due to the suitable ecological niches, particularly thick undergrowth available in these habitats. Lalang habitat appeared to be suitable for *M. malayanus* but not in the case of the other 3 species of land slugs. The higher infectivity rate among *M. malayanus* and *G. peguensis* could be due to the better suitability to the parasite of these 2 species than that of *L. alte* and *Lemperula* sp. This observation confirmed the findings of Lim *et al.* (1965), Lim and Heyneman (1965), and Lim and Ungku Dato Omar-Ahmad (1969).

Among the land snails, *A. fulica* was found to be the commonest and most widely distributed throughout all the habitats studied. *M. resplendens* and *S. octona*, although abundant were more restricted to specific habitats. The density of *M. resplendens* in oilpalm, in particular, could be due to the suitability of the habitat for this snail. Infectivity was found to be highest in *M. resplendens*, and suggests that this snail is a more suitable host of the parasite than *A. fulica* which had a much lower prevalence of infection. The present findings of high prevalence of natural infection in *M. resplendens* agreed with Lim and Heyneman (1965), while that of *A. fulica* was contrary to the findings of Bisseru and Verghese (1970).

Of the aquatic snails, *P. scutata*, *I. exustus* and *L. rubiginosa* are abundant in ricefields, while *B. ingallsiana* although common, are not abundant, confirming previous findings by Lim *et al.* (1977). Higher infectivity rates in *P. scutata* and *L. rubiginosa* indicates a greater susceptibility to the parasite than *I. exustus* and *B. ingallsiana;* the latter 2 species showed a lower prevalence of natural infection.

Among the house rats, *R. norvegicus* was found to have a higher infection rate with *A. malaysiensis* than *R. r. diardii*. The high infectivity rate in the former species could be due to the different behavioral pattern and ecological niches of the species than that of *R. r. diardii*. The latter is strictly a house rat confined more to indoors while *R. norvegicus* is equally common in indoors as well as outdoors, providing a greater chance for this species to come in contact with the molluscan intermediate hosts. Of the field rats, *R. tiomanicus* had a higher infection rate than *R. argentiventer* and *R. exulans*. Again, this could be due to differences in the food habits. Lim (1966) in his experimental field and laboratory studies on 4 species of house and field rats and 8 species of land snails and slugs, showed that *M. malayanus* and *M. resplendens* were preferred food, particularly, *M. resplendens*. *R. tiomanicus* was found to consume more of these snails than the other 3 species of rodents under experimental conditions. Thus, the present observation of *R. tiomanicus* with high infectivity rates could be attributed to the food preference and also to the easy assessibility of the molluscan intermediate hosts. The findings of larval stages in *S. murinus*, is evidence that this animal may feed on infected molluscs. However, no adult worms were ever recovered from this house shrew, supporting the findings of Lim *et al.* (1965) that this species is not a suitable host for the parasite. The low prevalence of natural infection among the forest rats in view of the abundance and high infection rates of the molluscan hosts, indicates high probability of resistance among these rats which concurs with findings of Lim *et al.* (1965).

The distribution of *A. malaysiensis* in Malaysia, indicates that a favourable habitat for the 2 key intermediate hosts *(M. malayanus* and *M. resplendens)* is the determining factor: where density of populations of both intermediate and final hosts is high, as in oil-palm and scrub (Fig. 1). Lalang fields are subjected to frequent grass-fires, and neither rodent nor molluscan hosts can remain for long periods in such a habitat, a disruption inimical to the maintenance of the *Angiostrongylus* life-cycle. The transmission cycle of the parasite in town is minimal due to the fact that the activities of urban rodents are more confined to houses, and the chances of contact with the intermediate hosts outside houses are restricted. In rubber habitats, good breeding grounds for both molluscs and rats in the weedy litter, provide excellent environment for direct food-chain to sustain the parasite. However, the low prevalence of natural infections in both hosts could be due to periodic clearing and weeding of the rubber estates. Similar to the lalang habitats, the life-cycle of *Angiostrongylus* is also disrupted in rubber habitats.

In the ricefield habitats, freshwater molluscs are also involved in the life-cycle, and apparently are important additional intermediate hosts. Successful feeding experiments with these aquatic hosts in the laboratory confirmed this (Lim *et at.*, 1977) and the presence of shells inside burrows of ricefield rats *(R. argentiventer)* offers further evidence. In Thailand freshwater snails, particularly *Pila* spp. were found to be important intermediate hosts of *A. cantonensis*, the cause of eosinophilic meningoencephalitis in man in that country. (Harinasuta *et al.*, 1964; Crook *et al.*, 1968). In Taiwan (Formosa), *Cipangopaludina chinensis*, commonly found in ricefields, is also known to be a natural intermediate host of *A. cantonensis* (Chang *et al.*, 1968). In the present investigation *P. scutata* and *B. ingallsiana*, edible snails, common in ricefields in Malaysia, were found to harbor infective stage larvae of *A. malaysiensis* and were partly responsible for the infection of rats in ricefields. These species should therefore not be overlooked as a potential source of human infection in Malaysia.

Studies of the life-cycle of *A. malaysiensis* showed that there was no marked difference in various sizes of first-stage larvae between *A. malaysiensis* and *A. cantonensis*. The first and second moult of *A. malaysiensis* larvae in experimentally infected snails *(L. rubiginosa)* were observed 5-7 days and 12-16 days after infection, compared to 7-10 days and 12-16 days in *A. cantonensis* larvae in experimentally infected *Helicarion* sp. by Bhaibulaya (1975). The second-stage larvae of *A. malaysiensis* developed in the snail hosts at 5 days compared to 7 days for *A. cantonensis*. The delay in the moulting period and developing stage in *A. cantonensis* may, however, be due to different species of experimental snail hosts.

The rate of growth of *A. malaysiensis* larvae in experimentally infected snails in the present study was observed to be more rapid than *A. cantonensis.* Third-stage *A. malaysiensis* larvae were observed 13 days after infection and these larvae were found infective to laboratory rats. At 16 days all larvae recovered from experimentally infected snails were fully developed third-stage larvae. In *A. cantonensis,* however, infective stage larvae are not present until 21 days after infection. The differences between *A. malaysiensis* and *A. cantonensis* development may be due to the different species of intermediate hosts used.

In experimentally infected rats, the third moult of *A. malaysiensis* occurred 4-6 days after infection in the present study and is similar to *A. cantonensis* as observed by Bhaibulaya (1975). The fourth-stage larvae of *A. malaysiensis* recovered from the brain grew to twice the length of the third-stage at 6 days compared to 7 days for *A. cantonensis.* The male and female *A. malaysiensis* matured at 18 and 24 days compared to 28 and 35 days for *A. cantonensis.* First-stage larvae were found in lung tissues at 32 days and in feces at 35 days for *A. malaysiensis,* but in *A. cantonensis* first-stage larvae were first observed in feces 40-42 days after infection.

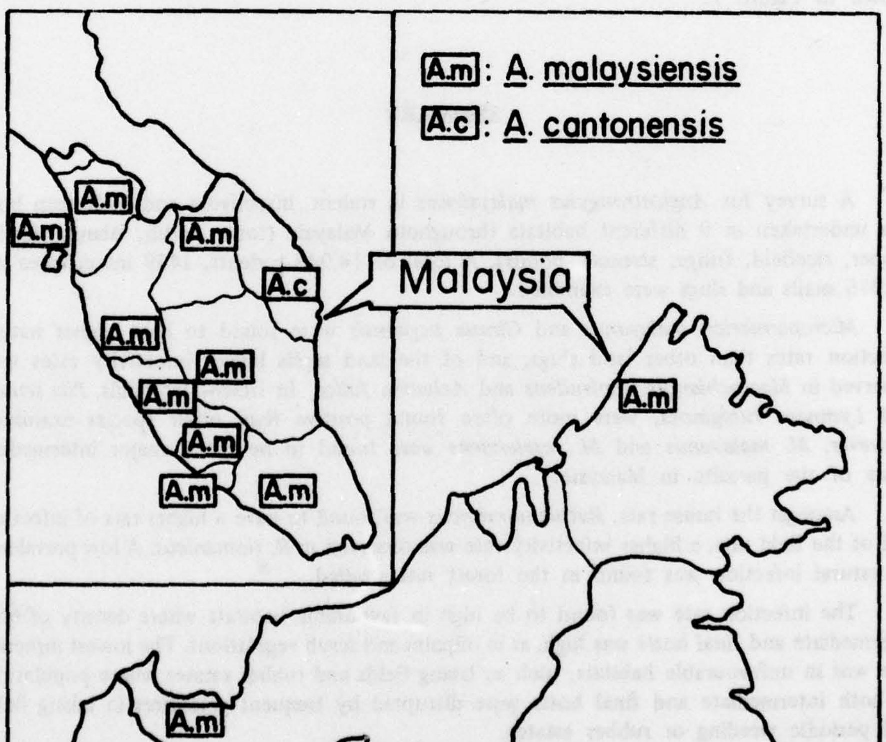

Figure 7. Distribution of *Angiostrongylus malaysiensis* and *Angiostrongylus cantonensis* in Malaysia.

In conclusion, the present investigation revealed that the distribuiton of *A. malaysiensis* in different habitats is influenced by numerous intermediate molluscan and final hosts throughout Malaysia. The present findings on the fully mature male and female *A. malaysiensis* concur with the description of the Malaysian rat lungworm by earlier workers (Lim *et al.*, 1965). This, however, does not proclude the occurrence of *A. cantonensis* in Malaysia. Indeed a single case of *A. cantonensis* has been reported in *R. exulans* from Trengganu by Lim (1975). Mixed infection of *A. malaysiensis* and *A. cantonensis* in the final host was observed in Thailand by Bhaibulaya and Techasophonmani (1972). Failure to recover *A. cantonensis* in field rats in northern, central and southern parts of Peninsular Malaysia and also from Sabah and Sarawak (Lim, 1967; 1973; 1974; Lim *et al.*, 1976), does not necessary mean that the parasite is restricted to the East Coast of Peninsular Malaysia (Lim, 1975). Further work should place emphasis on the taxonomic status of the rat lungworm found in a particular host in order to assertain the species found and also the occurrence of mixed infections (i.e. both *A. malaysiensis* and *A. cantonensis* occurring in the same host). The present status of the distribution of *A. malaysiensis* and *A. cantonensis* in Malaysia is shown in Figure 7.

SUMMARY

A survey for *Angiostrongylus malaysiensis* in rodent, insectivore and molluscan hosts was undertaken in 9 different habitats throughout Malaysia (town, scrub, lalang, oilpalm, rubber, ricefield, fringe, streams, ponds). A total of 14,948 rodents, 1439 insectivores and 21,016 snails and slugs were examined.

Microparmarion malayanus and *Girasia peguensis* were found to have higher natural infection rates than other land slugs, and of the land snails higher infectivity rates were observed in *Macrochlamys resplendens* and *Achatina fulica*. In freshwater snails, *Pila scutata* and *Lymnaea rubiginosa*, were more often found positive than other species examined. However, *M. malayanus* and *M. resplendens* were found to be the 2 major intermediate hosts of the parasite in Malaysia.

Amongst the house rats, *Rattus norvegicus* was found to have a higher rate of infection, and of the field rats, a higher infectivity rate was observed in *R. tiomanicus*. A low prevalence of natural infection was found in the forest rats studied.

The infection rate was found to be high in favourable habitats where density of both intermediate and final hosts was high, as in oilpalm and scrub vegetations. The lowest infection rate was in unfavourable habitats, such as lalang fields and rubber estates where populations of both intermediate and final hosts were disrupted by frequent grass-fires in lalang fields and periodic weeding or rubber estates.

The life-cycle of the parasite in all the habitats studied, except ricefields, is maintained chiefly by one species of land slug, *M. malayanus* and by one species of land snail, *M. resplendens*. In ricefields *Pila scutata* and *Lymnaea rubiginosa* are the main freshwater snails involved. Both edible freshwater snails, *Pila scutata* and *Bellamya ingallsiana* are probably important intermediate hosts in the transmission of human *Angiostrongylus*-

induced eosinophilic meningitis meningoencephalitis in Malaysia.

The life-cycle of the rat lungworm *A. malaysiensis* is reported for the first time in an experimental intermediate host, *L. rubiginosa,* and an experimental final host, a laboratory strain of *R. norvegicus.* The rate of growth of *A. malaysiensis* larvae in experimentally infected snails was observed to be more rapid than *A. cantonensis.* Slight differences in the growth rate between *A. malaysiensis* and *A. cantonensis* were also observed in laboratory rats. The male and female *A. malaysiensis* matured as early as 18 and 24 days compared to 28 and 35 days for *A. cantonensis.*

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GEOGRAPHICAL DISTRIBUTION OF *ANGIOSTRONGYLUS* AND ANGIOSTRONGYLIASIS IN THAILAND, INDO-CHINA AND AUSTRALIA

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THAILAND

In Thailand, Kwanmitra *et al.* (1957) reported 4 cases of eosinophilia in the cerebrospinal fluid and suspected one of the 4 cases was the result of gnathostomiasis. Subsequently ocular angiostrongyliasis (Prommindaroj *et al.*, 1962; Ketsuwan and Pradatsundarasar, 1966) and eosinophilic meningoencephalitis caused by *Angiostrongylus cantonensis* were reported (Tantibhedyangur, 1963; Benjapongse, 1964; Punyagupta, 1964; Buranasin *et al.*, 1965; Chularerk and Suyarnsethakorn, 1965; Jittayasothorn *et al.*, 1965; Hongladarom and Indarakoses, 1966; Tangchai *et al.*, 1967; Punyagupta *et al.*, 1975; Bunnag *et al.*, 1969; Nitidandhaprabhas *et al.*, 1975). Most of the cases reported were from northeastern Thailand.

Six species of rodent are reported to serve as natural definitive hosts of *A. cantonensis* namely: *Rattus norvegicus, R. rattus, R. berdmorei, Rattus* sp. *Bandicota indica* and *B. savilei*, and animals were found harbouring the parasite throughout most of the country (Harinasuta *et al.*, 1965; Crook *et al.*, 1968).

A. malaysiensis has been found singly or with *A. cantonensis* in *R. norvegicus* and *R. rattus* in the Central Thailand (Bhaibulaya and Techasophomani, 1972; Jeradit, 1977).

A history of consuming *"Pila"* spp. snails has been reported before the onset of eosinophilic meningitis (Tantibhedyangur, 1963) in many patients. Punyagupta (1964) found *Pila ampullacea* to be a natural intermediate host and Harinasuta *et al.* (1965) reported *P. turbinis, P. angelica, P. polita, P. gracilis* and *Achatina fulica* to be natural intermediate hosts. In addition, to the above mentioned molluscs, *Veronicella siamensis, Sarika resplendens, Hemiplecta siamensis* and *Melanoides tuberculata* were also found to be naturally infected with *A. cantonensis* (Crook *et al.*, 1968). Setasuban *et al.* (1968) added *P. scutata* and *Sinotiana martensiana* to the list. Although *A. fulica* is found infected with *A. cantonensis* in most parts of country *Pila* spp. are considered more important vectors to humans since they are habitually eaten raw by this.

A. malaysiensis larvae has been found alone or along with *A. cantonensis* in *A. fulica, P. ampullacea, P. pesmei* and *P. gracilis* (Jeradit, 1977).

INDO-CHINA

In Indo-china, meningoencephalitis and ocular angiostrongyliasis caused by *A. cantonensis* were reported from Vietnam and Laos (Jindrak and Alicata, 1965; Le-Van-Hou *et al.*, 1973; Fontan *et al.*, 1975). Among rodents and molluscs, only *A. cantonensis*, has been reported from *R. norvegicus* and *A. fulica* in Cambodia and Vietnam (Brumpt *et al.*, 1968; Nhuan and Hendricks, 1974).

In Thailand and Indo-china it appears that *R. norvegicus* and *A. fulica* are responsible for the distribution and maintainance of *A. cantonensis* in nature. *Pila* species of snails, however, seem to be more responsible for the transmission of the disease to man, particularly in Thailand.

AUSTRALIA

Although human angiostrongylid induced eosinophilic meningitis is not common in Australia the immunologic diagnoses of cases were made from Queensland (Bhaibulaya and Gutteridge, 1971; Gutteridge and Bhaibulaya, 1971) and an additional case has been referred to by Gutteridge (1971). Saltos *et al.* (1975) reported another probable case in 1975. None of these cases have been parasitologically confirmed. *A. cantonensis* has been known from rats in Australian since 1955 (Mackerras and Sandars, 1955) and an angiostrongylid with short spicules, later described as *A. mackerrasae* (Bhaibulaya, 1968), was also found in these animals. *R. norvegicus* was found to harbour either pure *A. cantonensis* or pure *A. mackerrasae.* Mixed infection between these two species in *R. norvegicus* was also found. *R. rattus* harboured only *A. cantonensis,* whereas *R. fuscipes* harboured only *A. mackerrasae* (Bhaibulaya, 1968). Although adult *A. cantonensis* was not found, immature adults were recovered from the central nervous system of the wallaby, *Macropus rufogriseus;* the first such report from a marsupial (Mckenzie *et al.*, 1978). The molluscs, *Deroceras laevae (Agriolimax laevis), Limax arborum, Onchidium sp.* and *Hericarion sp.* were reported as experimental intermediate host (Mackerras and Sandars, 1955; Bhaibulaya, 1968) for the parasite.

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ANGIOSTRONGYLUS CANTONENSIS IN THE PHILIPPINES: A REVIEW

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Although *Angiostrongylus cantonensis* was described in 1935 by Chen, the nematode was first demonstrated in the Philippines in 1965 by two groups working independently (De Leon and Saulog, 1965; Nishimura and Yogore, 1965). A number of surveys have since been undertaken to determine the presence of infection in rats and to identify possible molluscan intermediate hosts in different parts of the country. However, no special study has been done to define infection in humans although 4 cases with high numbers of eosinophiles in the spinal fluid were reported by Sison *et al.* (1951) and a suspected case of *A. cantonensis* induced eosinophilic meningoencephalitis was reported by Stransky and Joven (1964). Up to the present time no authenticated or parasitologically confirmed human cases of angiostrongyliasis have been reported.

DISTRIBUTION

From the available observations and based on ecologic similarities of the different regions of the Philippines, it is safe to assume that *A. cantonensis* is endemic in urban and rural areas of most parts of the Philippines (Fig. 1).

The initial demonstration of *A. cantonensis* by Nishimura and Yogoré (1965) and De Leon and Saulog (1965) were from rats caught in Metropolitan Manila. Similarly, Latonio (1968) and Salazar and Cabrera (1969) found infected rats in Manila and Quezon City. In addition, Latonio (1968) found infected rats in the provinces of Rizal, Cavite, Laguna and Batangas in the Southern Tagalog Region of the Island of Luzon; in the provinces of Pampanga, Bulacan, Bataan and Nueva Ecija in Central Luzon; and in Baguio City and the province of Isabela in Northern Luzon. Westerlund and Chamberlain (1969) reported finding the parasite in wild rats in the Mountain Province in Northern Luzon. Latonio (1968) reported the nematode in rats in the Visayan Region such as Leyte, and Guerrero and Guerrero (1972) found the parasite in commensal rats in Dumaguete City, Negros Oriental.

No organized surveys have been undertaken in provinces of Mindanao but a few

A. fulica collected and examined in March 1976 from Bislig were found with infective stage *A. cantonensis* larvae (Cross, 1978 personal communication). As stated earlier in view of the ecologic similarities of the regions of the Philippines, it is highly probable that *A. cantonensis* is enzootic throughout the Archipelago.

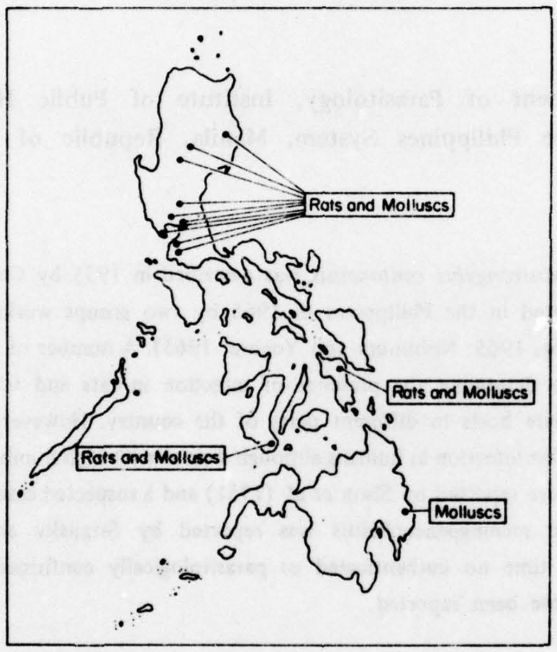

Figure 1. Locations in the Philippines where rodents and molluscs have been found infected with *Angiostrongylus cantonensis*.

HOSTS

Different species of the genus *Rattus* have been demonstrated as natural definitive hosts. These include *Rattus rattus, R. norvegicus, R. rattus mindanenis, R. exulans, R. everetti* and *R. lutiventus* (De Leon and Saulog, 1965; Nishimura and Yogore, 1965; Latonio *et al.*, 1968; Westerlund and Chamberlain, 1969; Guerrero and Guerrero, 1972). The last 3 species were caught in the wilderness of Mountain Province (Westerlund and Chamberlain, 1969).

Molluscan intermediate hosts of *A. cantonensis* identified in the Philippines as natural hosts include species of snails and slugs. Natural infections have been found in the giant African snail, *Achatina fulica* (Arambulo and Yogore, 1966; Latonio *et al.*, 1968; Salazar and Cabrera, 1969; Westerlund and Chamberlain, 1969; Guerrero and Guerrero, 1972) and in the jungle terrestrial snails, *Hemiplecta sagittifera, Helicostyla mactrostoma, Cyclophorus* sp., and *Chlorea fibula* (Westerlund and Chamberlain, 1969). *Pila luzonica, Vivipara* sp., and *Thiara* sp. were examined but not found naturally infected (Salazar and Cabrera, 1969; Westerlund and Chamberlain, 1969). The black slug, *Laevicaulis alte,* and the brown slug, *Imesinia plebe,* have also been found naturally infected (Salazar and Cabrera, 1969).

HUMAN INFECTION

Earlier reports by Sison *et al.* (1951) and Stransky and Joven (1964) of patients with eosinophilic meningitis suggest that the cases may have been angiostrongylid induced, however, none of the cases were parasitologically proven. The clinical manifestations were compatible with those described by Horio and Alicata (1961) and Alicata (1964). The clinical presentation of meningoencephalitis in the 5 cases in the Philippines was typical and all patients recovered spontaneously. It the cases reported by Sison *et al.* (1951) eosinophile levels in the cerebrospinal fluid ranged from 67% to 96% while in the case reported by Stransky and Joven (1964) eosinophilic pleocytosis was 87%. Sison *et al.* (1951) did not consider *A. cantonensis* as the etiologic agent but Stransky and Joven (1964) strongly suspected the parasite as the cause of the illness.

It is routine practice in most hospitals in the Philippines to examine cerebrospinal fluid for cellular contents differentially in suspected cases of meningoencephalitis. In view of this, it may be said with some degree of confidence, that angiostrongylid induced eosionphilic meningitis or meningoencephalitis is rare in the Philippines. This may be explained by the eating habits of most of the people since few eat snails uncooked.

SUMMARY

In the Philippine Island the definitive host for *Angiostrongylus cantonensis* are reported as *Rattus rattus, R. norvegicus, R. rattus mindanenis, R. exulaus, R. everetti* and *R. lutiventus.* The molluscan intermediate host for the parasite are *Achatina fulica, Hemiplecta sagittifer, Helicostyla mactrostoma, Cyclophorus* sp., *Chlorea* sp., *Laevicaulis alte* and *Imesinia plebe.* No parasitologically confirmed human cases of angiostrongyliasis have been reported but 4 cases of eosinophilic pleocytosis and one patient with eosinophilic meningoencephalitis are suspected to have been angiostrongylid induced.

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ANGIOSTRONGYLIASIS AND EOSINOPHILIC MENINGITIS ON TAIWAN: A REVIEW

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In areas of the Pacific and in Southeast Asia, eosinophilic meningitis or meningoencephalitis is caused mainly by invasion of the human central nervous system by the rat lungworm, *Angiostrongylus cantonensis.* The parasite normally inhabits the pulmonary arteries of rodents that become infected by eating mulluscan intermediate hosts, such as land snails and slugs, harboring the infective third-stage larvae. Human infection is also from the ingestion of molluscs infected with third-stage larvae. The larvae migrate into the brain, spinal cord, eyes and cause disease. The first case of angiostrongyliasis was discovered by Nomura and Lin in October 1944 in Tainan, South Taiwan, from which more than 10 actively moving immature adult worms were recovered in the cerebrospinal fluid (CSF) of a 15-year-old boy suspected as having meningitis. Since 1944, more than 250 cases of eosinophilic meningitis or meningoencephalitis have been found on Taiwan.

This paper presents a review of epidemiological studies on the definitive intermediate and paratenic hosts and human cases of *A. cantonensis* on Taiwan up to June 1978. The mode of infection to man is also discussed.

DEFINITIVE HOSTS

The first report of *A. cantonensis* on Taiwan was published by Matsumoto (1937) in which he described the morphology of the nematode collected from the lungs of wild rats captured in Hualien on the east coast of the island. The worm was subsequently identified as a new species, *Hemostrongylus ratti,* by Yokogawa (1937).

Table 1 indicates results of rodent surveys carried out on Taiwan and its offshore islands by various investigators for *A. cantonensis.* Kuntz and Myers (1964) reported on studies done between 1957 and 1962 and found 99 of 1,650 rodents naturally infected with this parasite. The species of rodents found infected on Taiwan proper were *Bandicota indica nemorivaga, Rattus coxinga coxinga, R. losea, R. norvegicus* and *R. rattus* subsp., and from Orchid Island *R. rattus mindanensis.* These species were all captured in areas below 1,500 feet in altitude.

Table 1

Surveys of rodents for *Angiostrongylus cantonensis* on Taiwan and its offshore islands

Species (Area sample collected)	No. examined	% infected	Reference
Rattus coxinga coxinga (Various areas on Taiwan)	47	4*	Kuntz *et al.*, 1964
Rattus coxinga coxinga (Various areas on Taiwan)	3	33	Cross, 1967
R. losea (Various areas on Taiwan)	172	8	Kuntz *et al.*, 1964
R. norvegicus (Penghu+)	15	0	Kuntz *et al.*, 1964
R. norvegicus (Various areas on Taiwan)	328	8	Kuntz *et al.*, 1964
R. norvegicus (Various areas on Taiwan)	101	9	Cross, 1967
R. norvegicus (Penghu+)	35	0	Chen, 1972
R. norvegicus (Kaohsiung County)	5	0	Yii *et al.*, 1975
R. norvegicus (Penghu+)	7	71	Cross *et al.*, 1976
R. norvegicus (Various areas on Taiwan)	47	38	Otsuru, 1977
R. rattus subsp. (Penghu+)	16	0	Kuntz *et al.*, 1964
R. rattus subsp. (Various areas on Taiwan)	792	3	Kuntz *et al.*, 1964
R. rattus subsp. (Various areas on Taiwan)	250	9	Cross, 1967
R. rattus subsp. (Penghu+)	11	0	Chen, 1972
R. rattus subsp. (Kaohsiung County)	6	3	Yii *et al.*, 1975
R. rattus subsp. (Various areas on Taiwan)	47	36	Otsuru, 1977
R. rattus mindanensis (Lanyu++)	64	2	Kuntz *et al.*, 1964
R. rattus rufescens (Penghu+)	14	14	Cross and Van Peenen, 1976
R. culturutus (Various areas on Taiwan)	32	0	Kuntz *et al.*, 1964
Bandicota indica nemorivaga (Various areas on Taiwan)	130	25	Kuntz *et al.*, 1964
Bandicota indica nemorivaga (Various areas on Taiwan)	20	50	Cross, 1967
Bandicota indica nemorivaga (Kaohsiung County)	15	53	Yii *et al.*, 1975
Bandicota indica nemorivaga (Various areas on Taiwan)	24	96	Otsuru, 1977
Apodemus agrarius insulaemus (Various areas on Taiwan)	11	0	Kuntz *et al.*, 1964
Apodemus semotus (Various areas on Taiwan)	57	0	Kuntz *et al.*, 1964
Micromys minutus takasagoensis (Various areas on Taiwan)	11	0	Kuntz *et al.*, 1964
Mus formosanus (Various areas on Taiwan)	10	0	Kuntz *et al.*, 1964
Mus musculus subsp. (Various areas on Taiwan)	47	0	Kuntz *et al.*, 1964
Mus musculus subsp. (Various areas on Taiwan)	10	0	Cross, 1967
Mus sp. (Various areas on Taiwan)	1	0	Otsuru, 1977
Suncus murinus (Various areas on Taiwan)	17	0	Cross, 1967
Suncus murinus (Various areas on Taiwan)	27	0	Otsuru, 1977

* Rounded to nearest whole number.

\+ Penghu County or Pescadores Islands situated 40 miles to the west of Taiwan.

++ Lanyu or Orchid Island situated 50 miles to the southeast of Taiwan. (See Figure 1)

Cross (1967), in a study done in conjunction with a survey for leptospirosis in Taiwan rodents, reported infections in 4 of 6 species of rodents among 400 animals examined from various parts of Taiwan. Infections were found in approximately 9%, 9%, 33%, and 55%, respectively, of *R. norvegicus*, *R. rattus* subsp., *R. coxinga coxinga* and *B. indica nemorivaga*.

During a series of studies on eosinophilic meningitis in southern Taiwan undertaken at the Kaohsiung Medical College, Yii *et al.* (1975) reported recovery of *A. cantonensis* from *R. rattus* subsp. and *B. indica nemorivaga*. The animals were trapped in the village of a patient whose cerebrospinal fluid contained *A. cantonensis* larvae. Since 1972, Otsuru (1977) and his colleagues have carried out surveys for angiostrongyliasis on Taiwan proper and reported finding *A. cantonensis* most commonly in *R. norvegicus*, *R. rattus*, and *B. indica nemorivaga*. These authors (Otsuru *et al.*, 1976) reported approximately 9.8 worms per infected rat captured in Taipei City.

Rodents on the Pescadores Islands had been examined by Kuntz and Myers (1964) and by Chen (1972), but they failed to recover the parasite. In 1976, however, Cross and Van Peenen while studying rats for scrub typhus in 5 areas of the Islands found *R. norvegicus* from Kung-Pei-Shan area and *R. rattus rufescens* from Ching-An area infected with *A. cantonensis*.

Many other species of rodents, such as *Apodemus agrarius insulaemus*, *A. semotus*, *Micromys minutus takasagoensis*, *Mus formosanus*, *M. musculus* subsp., *R. culturatus*, *M. musculus*, *Suncus murinus* and *Mus* sp., were examined, but none were found harboring the parasite.

Up to the present, 7 species of rodents have been found with *A. cantonensis* infection: *R. coxinga coxinga*, *R. losea*, *R. norvegicus*, *R. rattus* subsp., *B. indica nemorivaga* on Taiwan proper, *R. rattus mindanensis* on Lanyu or Orchid Island and *R. norvegicus* and *R. rattus rufescens* on Penghu or Pescadores Islands. The infection rates among rodents according to species and the localities are variable. The rate in bandicoots seems to be the highest, always in excess of 50%.

In experimental studies with the Formosan mongoose, *Herpestes urva*, Wood (1965) reported the animal susceptible to infection when given third-stage larvae of *A. cantonensis*. In later studies, however, Cross *et al.* (1970) using the same strain of *A. cantonensis*, failed to find the parasite in any of 13 mongooses experimentally exposed to infection.

INTERMEDIATE AND PARATENIC HOSTS

Studies on the intermediate and paratenic hosts for *A. cantonensis* on Taiwan have been carried out by several workers and results are listed in Table 2. Five molluscan species (2 species of land snails, *Achatina fulica* and *Bradybaena similaris*, one species of aquatic snail, *Cipangopaludina chinensis*, and 2 species of slugs, *Laevicaulis alte* and *Vaginulus plebeius)* are reported to serve as the natural intermediate hosts and one planarian species and 2 species of frogs *(Rana tigrina* and *R. plancyi)* are known to be natural paratenic hosts.

Table 2

Intermediate and paratenic hosts of *Angiostrongylus cantonensis* on Taiwan

Species (Area sample collected)	No. examined	% infected	Reference
Intermediate Hosts			
Snails			
Achatina fulica (Taipei City)	83	+	Chiu, 1964
Achatina fulica (Taitung County)	63	32*	Cross, 1967
Achatina fulica (Pingtung County)	241	28	Chen *et al.*, 1971
Achatina fulica (Pingtung County)	67	61	Wen, 1973
Achatina fulica (Various areas on Taiwan)	500	26	Otsuru, 1977
Bradybaena similaris (Taipei City)	218	+	Chiu, 1964
Cipangopaludina chinensis (Miaoli County)		+	Chang and Cross, 1966
Cipangopaludina chinensis (Ilan City & Pingtung County)	119	2	Otsuru *et al.*, 1976
Slugs			
Laevicaulis alte	14	43	Otsuru, 1977
Vaginulus plebeius (Taipei City; Taitung County)		+	Cross, 1967
Unidentified Slugs (Kaohsiung County)		10	Hsieh, 1967
Paratenic Hosts			
Planaria sp. (Taipei Hsien)		+	Cross, 1967
Frogs			
Rana tigrina	11	18	Otsuru, 1977
Rana plancyi	7	29	Otsuru, 1977

* Rounded to nearest whole number.

\+ Positive.

Among the intermediate hosts, *A. fulica,* plays the most important role for *A. cantonensis* infection to man on Taiwan. In reviewing the history on the importation and breeding of this giant African snail, Ezaki and Takahashi (1942) reported that the snail was first introduced to Taiwan by Shimojo, a Japanese technician. He brought 20 snails from Singapore to Taipei in January 1932, but all snails died from cold weather in the winter. In April 1933, he carried 12 snails to Taipei and cultivated them successfully. The snails bred enormously and spread throughout the entire island. Since the snail was harmful to vegetation and agricultural products, further importation was prohibited in 1936. The snail.

however, was well established on the island by that time.

Although the first human infection of this parasite was found in 1944 by Nomura and Lin, there were no further reports on the snail hosts of *A. cantonensis* on Taiwan until Chiu (1964) first recovered the larvae from *A. fulica* and *B. similaris* collected in Taipei City. Cross (1967) later found larvae in 20 (32%) of 63 *A. fulica* collected from Taitung County, along the east coast, where a case of angiostrongyliasis was found.

Table 3

Larvae of *Angiostrongylus cantonensis* in *Achatina fulica* by size (Chen *et al.*, 1971)

Size of snail (mm)	No. examined	Infected No.	Infected %	Average no. of larvae per infected snail
10 – 19	33	0	0	0
20 – 30	102	12	12	42
40 – 59	75	35	47	347
60 or more	31	20	65	454
TOTAL	241	67	28	324

Chen *et al.* (1971), in a biological study of *A. fulica*, found 67 (28%) of 241 snails collected form Santimen, an aboriginal village of Pingtung County, infected with larvae of *A. cantonensis*. They also correlated the infection rate and the intensity of the larval infection relative to the size of snails, i.e., the larger the size of the snail the higher the rate and the intensity of infection. As shown in Table 3, larvae were not recovered from snails with a length of less than 20 mm. The rates increased, however, from 12% to 65% and the average numbers of larvae per infected snail increased from 42 to 454 as the proporitonal size of snails increased from 20 mm to 60 mm or more. In the same report, they (Chen *et al.*, 1971) observed the seasonal fluctuation of snails in the field. They collected snails once a month for a one-year period from a defined garbage area about a 20 square meters in size. Snails were not seen in January and February, probably due to hibernation, but began to appear in March and were more active in May to September coinciding with the rainy season on Taiwan (Table 4).

Table 4

Monthly collection of *Achatina fulica* from a garbage disposal area (20 m^2) in 1970

(Chen *et al.*, 1971)

Month of collection	No. of snails* collected
Jan	0
Feb	0
Mar	35
Apr	89
May	263
Jun	485 and some young snails
Jul	564 and nemerous young snails
Aug	625 and numerous young snails
Sep	780 and numerous young snails
Oct	251 and numerous young snails
Nov	196 and numerous young snails
Dec	46 and some young snails

* The length of the snail was not less than 10 mm.

In the study of the distribution patterns of *A. cantonensis* larvae in *A. fulica*, Wen (1973) found that 41 (61%) of 67 snails collected from Pingtung county infected. He also reported finding 17,098 (56%) of 30,494 larvae in the lungs; the most densely infected snail organ. Wen (1977a; b) also reported that first-stage larvae passed through the respiratory pore to the lung of *A. fulica* and developed into the second- and third-stage larvae on the 7th and 17th days, respectively, after infection. The larvae developed faster in the lung than in the other organs of *A. fulica*.

In southern Taiwan, Yii *et al.* (1975) reported the recovery of *A. cantonensis* infective stage larvae from *A. fulica* collected in areas where cases of human eosinophilic meningitis occurred and from the snail meat served at food stands in towns and cities. The larvae in cooked snails, however, were dead. Otsuru (1977) and his colleagues examined 500 *A. fulica* collected from several localities on Taiwan and found third-stage *A. cantonensis* larvae in 26%.

Chen *et al.* (1974b) carried out a study to determine the intensity of infection. He collected 40 *A. fulica* from each of 8 localities. Snails collected from Taiwu, Pingtung County were the most heavily infected with an average of 405 larvae per snail.

A. fulica on Pescadores Islands were found to be negative for *A. cantonensis* by Chen (1972) and Wen (1977a), however, Cross and Van Peenen (1976) recovered the parasites from rodents in 2 areas on the islands and also found infected *A. fulica* in the same locations (Cross, 1978, personal communication).

Cipangopaludina chinensis, the Chinese rice paddy snail, was found to be a natural intermediate host for *A. cantonensis* on Taiwan by Chang and Cross (1966). They fed larvae obtained from these aquatic snails to laboratory rats and recovered adult *A. cantonensis.*

Several other species of freshwater snails had been examined for the presence of *A. cantonensis* larvae on Taiwan. Experimentally, Chang *et al.* (1968) recovered third-stage larvae from the aquatic snails, *Sinotaia quadrata, Lymnea swinhoe* and *Segmentina hemispherula*, after exposure to first-stage larvae. However, these snails have not been found naturally infected.

Cross (1967) found that *Semisulcospira libertina*, an intermediate host for *Paragonimus westermani* on Taiwan, to be resistant to infection and Otsuru *et al.* (1976) examined 126 *Semisulcospira* sp. collected from southern Taiwan and none were found naturally infected wi. 4. *cantonensis* larvae.

Two species of slugs were found naturally infected with the larvae of *A. cantonensis.* Cross (1967) recovered larvae from *Vaginulus plebeius* and Otsuru (1977) reported an infection rate of 43% in *Laevicaulis alte.* Hsieh (1967) in Kaohsiung County examined an unidentified species of slug and found larvae of the parasite.

A few studies of paratenic hosts of *A. cantonensis* on Taiwan have been undertaken. Cross (1967) reported planaria to naturally harbor larval stages and Otsuru (1977) found 2 species of frogs infected with the third-stage larvae. The infection rate for *R. tigrina* was 18%, and 29% for *R. plancyi.* Thirty-five toads, *Bufo melanostictus*, were also examined but larvae were not found. Otsuru *et al.* (1976) examined 6 species of freshwater shrimp and crabs, known paratenic hosts elsewhere, but none were infected.

HUMAN CASES

Most human cases of angiostrongyliasis and eosinophilic meningitis have occurred on the Pacific Islands and in Southeast Asia. The known cases on Taiwan until June 1978, including unpublished cases, are 259. Table 5 presents a summary of these cases.

Table 5

Cases of eosinophilic meningitis or meningoencephalitis on Taiwan from 1944 to 1978

Year	Number cases	Cases with worms	Recovered from	Reported by
1944	1+(1)	1	CSF	Nomura and Lin, 1945
1963	1	1	Eye	Hsieh, 1967
1964	1	1	Eye	Huang *et al.*, 1964
1965	1	1	CSF	Cross, 1967 (Case No. 3)
Year Unknown	1	1	CSF	Cross, 1967 (Case No. 4)
1965	3+(1)	0		Cross, 1967 (Case Nos. 5, 6, 7)
1966	1	1	CSF	Chen *et al.*, 1966
1966	1	0		Chang *et al.*, 1966
1966	3	1	CSF	Cross, 1967 (Case Nos. 10, 11, 12)
1967	3	0		Wang *et al.*, 1967
1967	2	1	CSF	Tai *et al.*, 1967
1967	5	2	CSF	Chen, 1967
1968-1969	125+(4)	9	CSF*	Yii *et al.*, 1968; Yii *et al.*, 1975; Yii, 1976
1970-1973	9	0		Chuang *et al.*, 1973
1971	1	0		Chiang, 1972
1973	1	0		Liang *et al.*, 1973
1970-1974	16	1	CSF	Pingtung Christian Hospital**
1975	2	0		Lin *et al.*, 1976
1970-1976	30+(1)	1	CSF	Cheng and Chen, 1976
1976	1	1	CSF	Lin, 1976 (Mackay Memorial Hospital)
1973-1977	22	0		Taitung Holy Cross Hospital**
1972-1977	20	0		Hwalien Mennonite Christian Hospital**
1977	1	1	CSF	Su, 1977 (Provincial Kaohsiung Hospital)
1977	2	0		National Taiwan University Hospital**

Table 5 (Continued)

Year	Number cases	Cases with worms	Recovered from	Reported by
1977	1	1	CSF	Chang-Gung Memorial Hospital••
1977-1978	2	1	CSF	Cheng, 1978 (Cheng's Pediatric Clinic)•••
1977-1978	2	1	CSF	Kaohsiung Medical College, 1978
1978	1+(1)	1	CSF	Hwang, 1978
TOTAL	259+(8)	27		

\+ Deaths (number)

• Worms recovered from cerebrospinal fluid, brain and lung in one patient at autopsy.

•• Cross, 1978, personal communication.

••• Cheng, 1978, personal communication.

The first case of human angiostrongyliasis reported anywhere was made by Nomura and Lin in March 1945, published in Japanese journal. Very little attention was paid to the report except for Hsieh's citation in an outline of parasitic zoonoses on Taiwan in 1959. Beaver and Rosen (1964) were informed and subsequently had the original article translated into English after the disease gained worldwide attention. The second case of angiostrongyliasis on Taiwan was reported by Huang *et al.* (1964) who recovered an immature worm from the anterior chamber of an 11-month-old girl. One year prior to the discovery of this case (October 1963), Hsieh (1967), on consultation at an eye clinic in Kaohsiung City, observed an actively moving worm in the eye of an adult female patient. The patient consented to surgery to remove the parasite, but the worm disappeared by the next day. This was a highly suspected case of ocular angiostrongyliasis.

Except for the 2 ocular cases of angiostrongyliasis occurring in 1963 and 1964, the remaining patients presented with meningitis or meningoencephalitis with eosinophilic pleocytoses. Young adult worms of *A. cantonensis* have been recovered from the CSF of 25 (10%) of the 257 cases. The geographical distribution of patients is plotted in Figure 1. Most of the patients were from southern and eastern Taiwan and only a few from nothern and central Taiwan. The distribution of patients by age group and sex is shown in Table 6. Most cases were children (80%) below 14 years of age but there is little difference in the distribution of cases by sex. No cases of eosinophilic meningitis have been reported from the offshore islands.

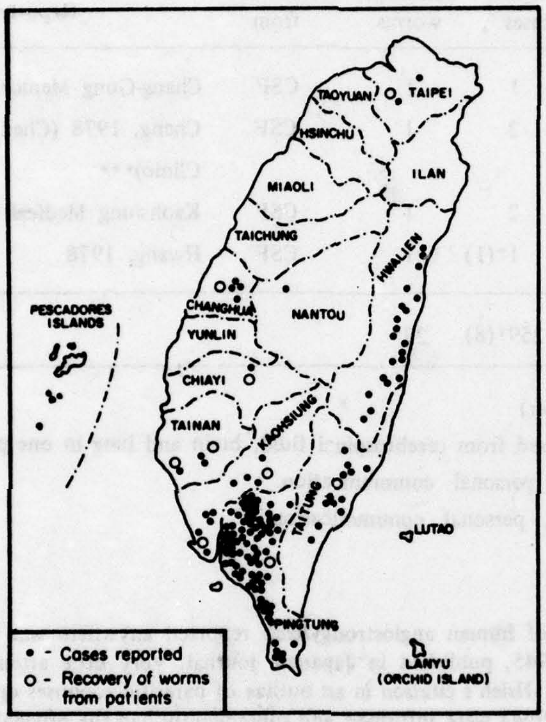

Figure 1. Map of Taiwan showing distribution of cases of eosinophilic meningitis or eosinophilic meningoencephalitis between 1944 and 1978.

Among 25 cases with worms present in the CSF, the parasites were recovered from 24 by lumbar puncture, and one at autopsy. Information relative to the days of onset of symptoms and numbers of worms recovered in the parasitologically confirmed cases is presented in Table 7. Although the days from onset of illness to discovery of worms varied from 4 to 30 days, in two-thirds of cases worms were found between the 10th and 30 days after onset of illness. In 5 cases, one reported by Nomura and Lin (1945), 3 by Yii (1976) and one case seen at the Kaohsiung Medical College (1978), 2 to 4 spinal tappings were performed on each patient and worms discovered at each tapping.

In epidemiological studies of eosinophilic meningitis on South Taiwan, Yii *et al.* (1975) correlated the distribution of 125 cases occurring in 1968 and 1969 by month of onset of first symptoms with monthly rainfall and found that more cases occurred in the rainy season when *A. fulica* was more active.

Table 6

Distribution of cases of eosinophilic meningitis or meningoencephalitis on Taiwan from 1944 to 1978 by sex and age group

Age (years)	Number of cases Male	Female	Totals	%
0 – 4	28	41	69	27
5 – 9	56	46	102	39
10 – 14	17	18	35	14
15 – 19	8	3	11	4
20 – 24	1	2	3	1
25 – 29	4	3	7	3
30 – 39	5	9	14	5
40 – 49	6	4	10	4
50+	1	2	3	1
Unknown	2	2	5*	2
TOTALS	128	130	259	100

* Sex of one case unknown.

Epidemiological surveys by clinical manifestations and laboratory findings for eosinophilic meningitis were reported by Cross (1967) and Yii (1976). The areas selected for surveys were localities where patients with the disease had lived. In a small village in Miaoli County, Cross (1967) reported that although the snail hosts, *A. fulica* and *C. chinensis* were found infected with *A. cantonensis* larvae, the rodent hosts examined, *R. rattus*, were negative. Over 100 individuals were examined but none had symptoms of meningitis although a large number of individuals had relatively high blood eosinophile levels. In another survey in Taitung County where a human case occurred no new human infections were found, but rodents, the giant African snails and slugs, *V. plebeius*, were found naturally infected with the parasite (Cross, 1967). Yii *et al.* (1975) performed diagnostic lumbar puncture on 54 persons with a history of headache in 2 aborigine villages of Pingtung County and detected 2 cases of eosinophilic meningitis.

Table 7

Days and numbers of worms recovered from the cerebrospinal fluid (CSF) after onset of symptom in 24 parasitologically confirmed cases

Age	Sex	Locality	Days from onset of symptom to recovery of worms	No. worms	Reference
15	M	Tainan City	10	10	Nomura and Lin, 1945
			11	*	
3	M	Pingtung County	?	4	Cross, 1967 (Case No. 3)
10	M	Taitung County	?	6	Cross, 1967 (Case No. 4)
10	M	Shihtze, Pingtung County	12	2	Chen *et al.*, 1966
3	F	Shihtze, Pingtung County	?	2	Cross, 1967 (Case No. 12)
7	F	Checheng, Pingtung County	4	3	Tai *et al.*, 1967
4	F	Fangliao, Pingtung County	9	1	Chen, 1967
7	F	Meishan, Chiayi County	?	15	Chen, 1967
7	M	Fangliao, Pingtung County	6	1	Yii, 1976
5	M	Anchao, Kaohsiung County	12	2	Yii, 1976
4	F	Fangliao, Pingtung County	12	4	Yii, 1976
8	M	Chaochow, Pingtung County	12	11	Yii, 1976
			15	2	
			19	1	
3	F	Pingtung City	16	1	Yii, 1976
			20	2	
3	M	Liukuey, Kaohsiung County	16	2	Yii, 1976
19	M	Fangshan, Pingtung County	5	1	Yii, 1976
			7	11	
			8	4	
7	F	Yenpu, Pingtung County	10	3	Yii, 1976
17	M	Pingtung City	?	*	Pingtung Christian Hospital+
1	F	Pingtung City	30	1	Cheng and Chen, 1976
2	M	Hsinhua, Tainan County	30	2	Lin, 1976 (Mackay Memorial Hospital)++
2	F	Kaohsiung County	24	1	Su, 1977 (Provincial Kaohsiung Hospital)++
2	F	Taishan, Taipei County	12	1	Chang-Gung Memorial Hospital+
2	M	Tungkang, Pingtung County	19	1	Cheng, 1978++
8	M	Linyuan, Kaohsiung County	22	1	Kaohsiung Medical College, 1978
			24	3	

Table 7 (Continued)

Age	Sex	Locality	Days from onset of symptom to recovery of worms	No. worms	Reference
			25	9	
			26	7	
2	M	Tienchung, Changhua County	?	+	Hwang, 1978

* Positive for worms, numbers not reported.

\+ Cross, 1978, personal communication.

++ Personal communication.

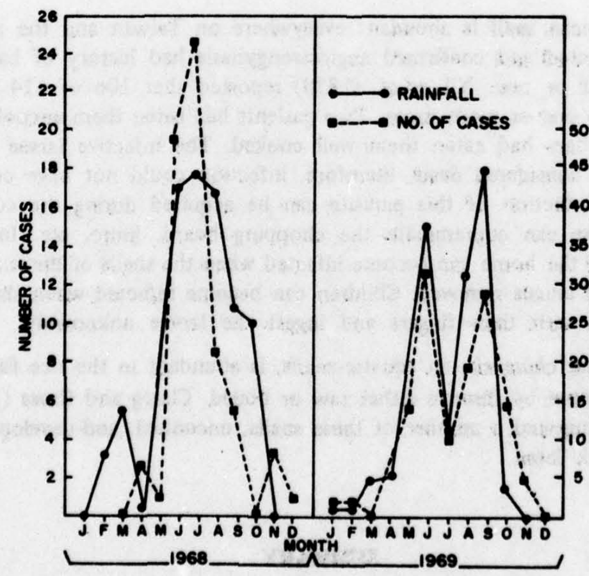

Figure 2. Distribution of cases of eosinophilic meningitis or meningoencephalitis by month of onset and rainfall occurring in Pingtung City from January 1968 to December 1969 (Yii *et al.*, 1975).

Seroepidemiological studies were also done to detect cases of angiostrongyliasis on Taiwan. Suzuki *et al.* (1975) carried out an indirect hemagglutination (IHA) test on 800 sera which were obtained from suspected cases of Japanese encephalitis. The majority of the IHA-positive sera were from persons from the southern area and southern half of the eastern area on Taiwan. Skin test surveys for human angiostrongyliasis were conducted by Chen *et al.* (1974a) on 1,459 persons residing in 11 localities on Taiwan and positive reaction rates among people living on the southern and eastern Taiwan were higher than those on the northern and central Taiwan. Suzuki *et al.* (1974) evaluated the skin test with a purified antigen prepared from adult *A. cantonensis* and suggested that the antibody responsible for positive reaction was present between 10 and 30 days after the onset of symptoms.

Clinical studies on angiostrongyliasis were done by Yii (1976) on 125 cases. The majority of patients had mild to moderate disease, but several had severe manifestations. Among 259 cases listed in Table 5, 8 patients died: one reported by Nomura and Lin (1945), one by Cross (1967), 4 by Yii (1976), one by Cheng and Chen (1976), and one by Hwang (1978). Sequalae such as blindness occurred in 6 cases: one case each reported by Huang *et al.* (1964), Chang and Cross (1966), one case by Liang *et al.* (1973) with bilateral blindness, and Yii (1976) reported 3 cases.

MODE OF INFECTION

The giant African snail is abundant everywhere on Taiwan and the majority of the patients with suspected and confirmed angiostrongyliasis had history of having eaten the snails either cooked or raw. Yii *et al.* (1975) reported that 106 of 114 patients (93%) had eaten the snails one or more times. Two patients had eaten them uncooked or partially cooked and the others had eaten them well cooked. The infective larvae in the cooked snails are generally considered dead, therefore, infection could not have occurred in this manner. However, infection of this parasite can be acquired during the cooking process; the third-stage larvae can contaminate the chopping board, knife, etc. In addition, the environment around the home can become infected when the shells of the snails are crushed and the viscera and mucus removed. Children can become infected when they handle contaminated materials with their fingers and ingest the larvae unknownly.

Cipangopaludina chinensis, an aquatic snails, is abundant in the rice fields on Taiwan and is commonly eaten by farmers either raw or boiled. Chang and Cross (1966) reported a patient who had ingested a number of these snails, uncooked, and developed eosinophilic meningitis one week later.

SUMMARY

A review on eosinophilic meningitis and angiostrongyliasis occurring on Taiwan from October 1944 until June 1978 is reported in this paper. Seven species of rodents were

found naturally infected with *Angiostrongylus cantonensis* and all infected rodents were captured in the areas below 1,500 feet altitude. *Bandicota indica nemorivaga* is commonly infected with the parasite with infection rates as high as 50%. Five species of molluscs have been reported to serve as the natural intermediate hosts and one species of planaria and 2 species of frogs have been found to be the natural paratenic hosts. *Achatina fulica* is the most widely distributed land snail and is the major scurce of *A. cantonensis* infection to man on Taiwan. The larger-sized snails were more commonly infected and contained larger numbers of larvae. The larvae were recovered in high numbers from the lung of snails. *A. fulica* appears more active in the rainy season.

A total of 259 confirmed and suspected cases of angiostrongyliasis has been reported on Taiwan. Among these cases, worms were removed from the eyes of 2 and found in the cerebrospinal fluid of 25 other patients. More cases were detected in southern and eastern Taiwan and 80% were in children of 14 years of age or less. The majority of patients had a history of having eaten snails and the most important route of infection seems to be in the preparation of the meat for cooking. Ingestion of raw aquatic snail, *C. chinensis*, is another means of infection on Taiwan.

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ANGIOSTRONGYLUS CANTONENSIS AND ANGIOSTRONGYLIASIS IN JAPAN

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There are several species of the Genus *Angiostrongylus* recorded from Rodentia. Of these, both *A. cantonensis* and *A. costaricensis* have recently been recognized as the cause of human disease. The former species is widely distributed in the Pacific Islands and Southeast Asia, while the latter is limited to Central America. They are both heterogenous nematodes which utilize mollusks and rats as intermediate and final hosts, respectively.

In the case of *A. cantonensis*, adult worms live in pulmonary arteries of wild rats; snails and slugs serve as intermediate hosts and human infection can usually be acquired by eating or handling the intermediate or paratenic hosts infected with the third-stage larvae. In man, the larvae migrate to the brain and meninges where they become young adults and produce reactions. *A. cantonensis* has been recognized as an etiologic agent of cases of eosinophilic meningoencephalitis in some areas of the Pacific Islands and Southeast Asia.

Generally speaking, animals in certain areas often include species intentionally or unintentionally imported from other areas. For example, in Japan, the mongoose and weasel are mammals imported intentionally to the Ryukyu Islands (Okinawa Islands and their southern islands), while the giant African snail, *Achatina fulica*, which causes great damage to vegetation was carelessly introduced to the Ryukyu and Amami Islands. Rats are well known as one of the most typical animals imported unintentionally. *Rattus rattus* and *R. norvegicus*, which are intimately related to human life, are the best examples of readily introduced species, and such animals carried by ships are too numerous to be mentioned. The slug, *Laevicaulis alte*, whose damage to vegetation has recently been uncovered in the Ryukyu and Amami Islands along with the giant African snails, was also probably introduced to these islands with cultivated plants. *A. cantonensis* is also believed to have been introduced to the Ryukyu and Amami Islands from Taiwan and/or the South Pacific Islands in relatively recent years. It is speculated in the Pacific and Asian tropical and subtropical zones that extention of the geographic range of *A. cantonensis* to adjacent regions has been through stowaway infected mollusks or rats.

The Southwest (Nansei) Islands (Fig. 1) including the Ryukyu and Amami Islands and the southern outlying island of Kyushu occupy the southern part of a long chain of islands in the Japan Archipelago. This chain forms an arc from the northeast to the southwest along the east coast of the Asian Continent, and is important in geomedicine. For example, in the spread of various infectious diseases to the Archipelago from Southeast Asia or the Asian Continent, the Southwest Islands have often played an important intermediate role. About 10 years after *A. cantonensis* was described in Canton, South China, the first case of human angiostrongyliasis was reported in a young man suffering from meningitis (Nomura and Lin, 1945) on Taiwan and since 1962 many additional cases have been seen on Taiwan. In the Ryukyu Islands, which are separated only by a narrow strait northeastward from Taiwan, at least 12 cases of the disease have been reported since 1969. *A. cantonensis* has been recovered from rats and slugs in the harborside areas of Tokyo and other main islands of Japan since 1969. The parasite seems to be well established in Japan and therefore, the disease is now considered one of the important public health problems in Japan, as it is in the Pacific Islands and Southeast Asia.

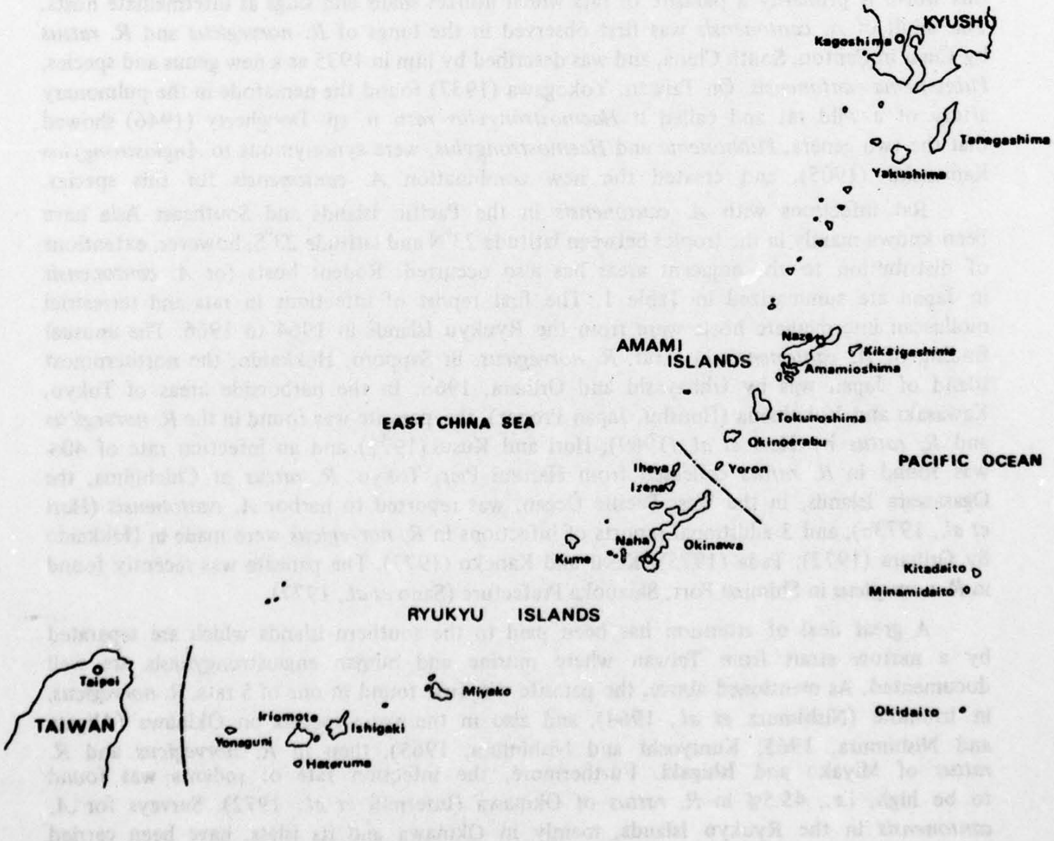

Figure 1. Map of the Southwest Islands (Nansei Islands) of Japan.

In this paper recent works on *A. cantonensis* and angiostrongyliasis done mainly in Japan are presented, in addition to studies done in neighboring Taiwan. The data quoted are derived from references collected as far as possible by the author and from the surveys in the Ryukyu Islands and neighboring regions carried out by his Department since 1972.

SURVEYS OF THE FINAL HOSTS

According to Skrjabin *et al.* (1952) and Chabaud (1972), *Angiostrongylus* is a genus belonging to Subfamily Angiostrongylinae, Family Filaroididae. There are about 26 known species of this genus recorded from a variety of hosts involving Rodentia, Carnivora, Insectivora, etc. Of these, *A. cantonensis* is the only species of public health importance known in the various islands of the Pacific Ocean and in Southeast Asia. As mentioned above, this worm is primarily a parasite of rats which utilizes snails and slugs as intermediate hosts. The adult of *A. cantonensis* was first observed in the lungs of *R. norvegicus* and *R. rattus* by Chen in Canton, South China, and was described by him in 1935 as a new genus and species, *Pulmonema cantonensis.* On Taiwan, Yokogawa (1937) found the nematode in the pulmonary artery of a wild rat and called it *Haemostrongylus ratti* n. sp. Dougherty (1946) showed that the two genera, *Pulmonema* and *Haemostrongylus,* were synonymous to *Angiostrongylus* Kamenskii (1905), and created the new combination *A. cantonensis* for this species.

Rat infections with *A. cantonensis* in the Pacific Islands and Southeast Asia have been known mainly in the tropics between latitude 23°N and latitude 23°S, however, extentions of distribution to the adjacent areas has also occurred, Rodent hosts for *A. cantonensis* in Japan are summarized in Table 1. The first report of infections in rats and terrestrial molluscan intermediate hosts were from the Ryukyu Islands in 1964 to 1966. The unusual finding of *A. cantonensis* in a rat, *R. norvegicus,* in Sapporo, Hokkaido, the northernmost island of Japan was by Ohbayashi and Orihara, 1968. In the harborside areas of Tokyo, Kawasaki and Yokohama (Honshu, Japan Proper), the parasite was found in the *R. norvegicus* and *R. rattus* by Hori *et al.* (1969); Hori and Kusui (1972), and an infection rate of 40% was found in *R. rattus* collected from Harumi Pier, Tokyo. *R. rattus* of Chichijima, the Ogasawara Islands, in the West Pacific Ocean, was reported to harbor *A. cantonensis* (Hori *et al.*, 1973b), and 3 additional reports of infections in *R. norvegicus* were made in Hokkaido by Orihara (1972); Tada (1975); Kasai and Kaneko (1977). The parasite was recently found in *R. norvegicus* in Shimizu Port, Shizuoka Prefecture (Sano *et al.*, 1977).

A great deal of attention has been paid to the southern islands which are separated by a narrow strait from Taiwan where murine and human angiostrongyliasis are well documented. As mentioned above, the parasite was first found in one of 5 rats, *R. norvegicus,* in Iriomote (Nishimura *et al.*, 1964), and also in the same species on Okinawa (Alicata and Nishimura, 1965; Kuniyoshi and Nishimura, 1965), then in *R. norvegicus* and *R. rattus* of Miyako and Ishigaki. Furthermore, the infection rate of rodents was found to be high, i.e., 45.5% in *R. rattus* of Okinawa (Intermill *et al.*, 1972). Surveys for *A. cantonensis* in the Ryukyu Islands, mainly in Okinawa and its islets, have been carried out by the Okinawa Prefectural Institute of Public Health since 1971. According to their

Table 1

Surveys of natural infections of rodents with *Angiostrongylus cantonensis* in Japan

Species	Locality	No. examined	No. infected (%)	Reference
	Positive findings			
Rattus norvegicus	Iriomote (Ryukyu)	5	1 (20.0)	Nishimura *et al.*, 1964
	Okinawa (Ryukyu)	55	8 (14.5)	Nishimura, 1966b
	Miyako (Ryukyu)	14	5 (35.7)	Nishimura, 1966b
	Ishigaki (Ryukyu)	28	4 (14.3)	Nishimura, 1966b
	Iriomote (Ryukyu)	23	2 (8.7)	Nishimura, 1966b
	Sapporo (Hokkaido)		1	Ohbayashi and Orihara, 1968
	Kawasaki (Honshu)	46	1 (2.2)	Hori *et al.*, 1969
	Yokohama (Honshu)	118	2 (1.6)	Hori *et al.*, 1969
	Sapporo (Hokkaido)		1	Orihara, 1972
	Harborside areas, Tokyo (Honshu)	190	22 (11.6)	Hori and Kusui, 1972
	Okinawa (Ryukyu)	115	45 (39.1)	Kuniyoshi *et al.*, 1972
	Okinawa (Ryukyu)	135	104 (77.0)	Intermill *et al.*, 1972
	Shinagawa and Harumi areas, Tokyo (Honshu)	79	11 (13.9)	Hori *et al.*, 1973a
	Haneda airport, Tokyo (Honshu)	63	1 (1.5)	Hori *et al.*, 1973a
	Takigawa (Hokkaido)		1	Tada, 1975
	Okinawa (Ryukyu)	139	48 (34.5)	Kishimoto and Asato, 1974
	Ishigaki (Ryukyu)	2	1 (50.0)	Kishimoto and Asato, 1974
	Kume (Ryukyu)	3	1 (33.3)	Kishimoto and Asato, 1974
	Izena (Ryukyu)	144	72 (50.0)	Asato and Kishimoto, 1976a
	Iriomote (Ryukyu)	1	1 (100)	Asato and Kishimoto, 1976a
	Yonaguni (Ryukyu)	2	1 (50.0)	Asato and Kishimoto, 1976a
	Shimizu port, Shizuoka (Honshu)	17	1 (5.9)	Sano *et al.*, 1977
	Sapporo (Hokkaido)	258	2 (0.8)	Kasai and Kaneko, 1977
	Yoron (Amami)	49	7 (14.3)	Yamashita *et al.*, 1978

Table 1 (Continued 1)

Species	Locality	No. examined	No. infected (%)	Reference
	Positive findings			
Rattus rattus	Okinawa (Ryukyu)		1	Kuniyoshi and Nishimura, 1965
	Okinawa (Ryukyu)	10	3 (30.0)	Nishimura, 1966b
	Okinawa (Ryukyu)	169	72 (42.6)	Intermill *et al.*, 1972
	Okinawa (Ryukyu)	150	32 (21.3)	Kuniyoshi *et al.*, 1972
	Harumi area, Tokyo (Honshu)	15	6 (40.0)	Hori and Kusui, 1972
	Chichijima (Ogasawara)	80	6 (7.5)	Hori *et al.*, 1973b
	Okinawa (Ryukyu)	204	35 (17.2)	Kishimoto and Asato, 1974
	Ishigaki (Ryukyu)	1	1 (100)	Kishimoto and Asato, 1974
	Izena (Ryukyu)	74	15 (20.3)	Asato and Kishimoto, 1976a
	Kume (Ryukyu)	10	1 (10.0)	Asato and Kishimoto, 1976a
	Iriomote (Ryukyu)	9	3 (33.3)	Asato and Kishimoto, 1976a
	Yonaguni (Ryukyu)	12	2 (16.7)	Asato and Kishimoto, 1976a
Suncus murinus riukiuanus	Okinawa (Ryukyu)	21	1 (4.8)	Intermill *et al.*, 1972
Rattus sp.	Okinawa (Ryukyu)	12	4 (33.3)	Alicata and Nishimura, 1965
	Negative findings			
Rattus norvegicus	Amamioshima (Amami)	43	–	Kawashima *et al.*, 1965
	Amamioshima (Amami)	27	–	Kamiya *et al.*, 1968
	Yoron (Amami)	11	–	Kamiya *et al.*, 1968
	Overseas ships of Yokohama and Kawasaki (Honshu)	22	–	Hori *et al.*, 1969
	Showajima, Tokyo (Honshu)	87	–	Hori *et al.*, 1973a
	Hahajima (Ogasawara)	35	–	Hori *et al.*, 1974
	Yokosuka (Honshu)	108	–	Otsuru *et al.*, 1978

Table 1 (Continued 2)

Species	Locality	No. examined	No. infected (%)	Reference
	Negative findings			
Rattus rattus	Iriomote (Ryukyu)	2	–	Nishimura *et al.*, 1964
	Amamioshima (Amami)	20	–	Kawashima *et al.*, 1965
	Amamioshima (Amami)	26	–	Kamiya *et al.*, 1968
	Yoron (Amami)	19	–	Kamiya *et al.*, 1968
	Overseas ships of Yokohama and Kawasaki (Honshu)	74	–	Hori *et al.*, 1969
	Shimizu port, Shizuoka (Honshu)	22	–	Sano *et al.*, 1977
Suncus murinus riukiuanus	Iriomote (Ryukyu)	2	–	Nishimura *et al.*, 1964
	Okinawa (Ryukyu)	90	–	Kuniyoshi *et al.*, 1972
	Okinawa (Ryukyu)	92	–	Kishimoto and Asato, 1974
	Kume (Ryukyu)	4	–	Asato and Kishimoto, 1976a
	Yonaguni (Ryukyu)	6	–	Asato and Kishimoto, 1976a
	Yoron (Amami)	7	–	Yamashita *et al.*, 1978
Tokudaia osimensis	Amamioshima (Amami)	1	–	Kamiya *et al.*, 1968
Mus musculus	Overseas ships of Yokohama and Kawasaki (Honshu)	9	–	Hori *et al.*, 1969
Mus musculus boninensis	Chichijima (Ogasawara)	4	–	Hori *et al.*, 1973b
	Hahajima (Ogasawara)	1	–	Hori *et al.*, 1974
Mus caroli	Okinawa (Ryukyu)	3	–	Kuniyoshi *et al.*, 1972
	Okinawa (Ryukyu)	8	–	Kishimoto and Asato, 1974
Herpestes edwardsii	Okinawa (Ryukyu)	2	–	Kuniyoshi *et al.*, 1972
	Okinawa (Ryukyu)	16	–	Asato and Kishimoto, 1976a

results (Kishimoto and Asato, 1974), 616 small mammals were examined, and the parasite was found in 18.4% of *R. rattus* and 37.1% of *R. norvegicus* examined. Similarly, on 2 islets of Okinawa, Izena and Kume, and on Ishigaki, Iriomote and Yonaguni, the infection rates were high. *Mus caroli, Suncus murinus riukiuanus* and *Herpestes edwardsii* were also examined but were negative (Table 1). Among 8 species or subspecies belonging to Muridae recorded in the Ryukyu Islands, *A. cantonensis* has been found in only *R. norvegicus* and *R. rattus*, with higher rates of infection in *R. norvegicus*. Similar results were obtained by Intermill *et al.* (1972). In Okinawa, *R. rattus* was trapped more often than *R. norvegicus* in the northern part, but this difference was not seen in the middle part, and the ratio reversed in the southern part of the island. *R. norvegicus* is known to live near human habitations and to be omnivorous, while *R. rattus* lives in the field and is more herbivorous. Recently, Yamashita *et al.* (1978) reported *R. norvegicus* to be infected with the parasite in Yoron, the southernmost island of the Amami Islands (Fig. 1). Adult worms had not been recovered by previous workers in the area.

Results of experimental murine infection carried out in Japan are summarized in Table 2. In oral infections, both *Praomys natalensis* and *Meriones unguiculatus* developed adult infections in the lungs (Yoshimura, 1973). Recently, Yamashita *et al.* (1975) obtained young adults measuring about 8 mm in body length from the brain of mice and guinea pigs 30 days after infection. In Okinawa, laboratory rats were infected by subcutaneous inoculation and by penetration of third-stage larvae through abraded and unabraded skin and adults were obtained from the lungs by each of the 3 alternate routes (Intermill *et al.*, 1972).

Table 2

Results of experimental infections of rodents with *Angiostrongylus cantonensis* in Japan

Species	Method of infection	No. examined	Stage of worm recovered	Reference
Laboratory rats	Prenatal	6	Not detected	Nishimura, 1965, 1966a
	Oral	32	Adult worms	Nishimura, 1965, 1966a
	Skin	23	Adult worms	Hori, 1969
	Skin	2	Adult worms	Intermill *et al.*, 1972
	Abraded skin	1	Adult worms	Intermill *et al.*, 1972
	Subcutaneous	1	Adult worms	Intermill *et al.*, 1972
	Oral	26	Adult worms	Intermill *et al.*, 1972
	Oral	10	Adult worms	Yoshimura, 1973
	Oral	6	Adult worms	Yamashita *et al.*, 1975
Praomys natalensis	Oral	12	Adult worms	Yoshimura, 1973
Meriones unguiculatus	Oral	9	Adult worms	Yoshimura, 1973
Mouse	Oral	4	Young adult worm in the brain	Yamashita *et al.*, 1975
Guinea pig	Oral	5	Young adult worm in the brain	Yamashita *et al.*, 1975

Table 3 shows data from epidemiological surveys on angiostrongyliasis on Taiwan Proper, which have been carried out by the members of the author's Department since 1972, together with the results of others. In the murine survey, *A. cantonensis* has been most commonly found in *R. norvegicus, R. rattus* and *Bandicota indica nemorivaga.* These results are similar to those reported by others on Taiwan (Kuntz and Myers, 1964; Cross, 1967).

Table 3

Surveys of natural infections of rodents, mollusks and frogs with *Angiostrongylus cantonensis* on Taiwan Proper

Species	No. examined	No. infected (%)	Reference
		Rodents	
Rattus norvegicus	328	(7.9)	Kuntz and Myers, 1964
	101	(9.0)	Cross, 1967
	47	18 (38.3)	Otsuru *et al.*, 1977a
Rattus rattus subsp.	792	(3.3)	Kuntz and Myers, 1964
	250	(9.2)	Cross, 1967
	47	17 (36.2)	Otsuru *et al.*, 1977a
Rattus coxinga coxinga	47	(4.2)	Kuntz and Myers, 1964
	3	(33.3)	Cross, 1967
Rattus losea	172	(7.6)	Kuntz and Myers, 1964
Bandicota indica nemorivaga	130	(25.3)	Kuntz and Myers, 1964
	20	(50.0)	Cross, 1967
	24	23 (95.8)	Otsuru *et al.*, 1977a
Suncus murinus	22	–	Otsuru *et al.*, 1977a
Mus sp.	1		Otsuru *et al.*, 1977a
		Mollusks	
Achatina fulica	83	positive	Chiu, 1964
	540	132 (24.4)	Otsuru *et al.*, 1977a
Bradybaena similaris	218	positive	Chiu, 1964
	136	–	Otsuru *et al.*, 1977a
Vaginulus plebeius		positive	Cross, 1967
Laevicaulis alte	14	6 (42.8)	Otsuru *et al.*, 1977a
Cipangopaludina chinensis		positive	Chang *et al.*, 1968
	1449	positive	Otsuru *et al.*, 1977a

Table 3 (Continued)

Species	No. examined	No. infected (%)	Reference
Semisulcospira sp.	126	–	Otsuru *et al.*, 1977a
Corbicula fluminea	312	–	Otsuru *et al.*, 1977a
Planaria		positive	Cross, 1967
		Frogs (paratenic hosts)	
Rana tigrina	94	3 (3.2)	Otsuru *et al.*, 1977a
Rana plancyi	28	6 (21.4)	Otsuru *et al.*, 1977a
Rana latouchi	14	3 (21.4)	Otsuru *et al.*, 1977a
Rana limnocharis	13	–	Otsuru *et al.*, 1977a
Bufo melanostrictus	12	1 (8.3)	Otsuru *et al.*, 1977a

The measurements of male and female adults parasitizing wild rats from various districts of Japan compared with those of Taiwan and Hawaii are shown in Table 4. It is known that their morphometric data bear resemblance to one another (Fig. 2 a, b).

In attempts to determine other diagnostic characters of *A. cantonensis*, morphological observations were made on cross-section of young adults (Ohmori and Suzuki, 1976). Various developing stages of worm were obtained from normal (albino rats) and abnormal host (guinea pigs) experimentally infected with the third-stage larvae of the Taiwan strain. Thirty days after infection, genital organs of both sexes of the parasite found in the lung of rats developed fully while the growth of worms in guinea pigs was retarded. The development of genital organs of both sexes of worms collected from the brains of the guinea pigs 29 days after infection was similar but somewhat retarded compared to worms collected from the brains of the rats 20 days after infection. Several common structures were found throughout each young adult *A. cantonensis* both in rats and guinea pigs after 15 days of infection as follows: (1) Submedian chords are usually seen except in the esophageal and caudal regions. (2) Body cavity contains many coelomocytes, seen in cross-sections (0-7/section). (3) Muscle cells in mid-body sections numbered 40-50 in the adult female and 35-45 in the adult male. (4) Sphicter muscles are seen at the junction between the oviduct and uterus of the female. The above characters, as well the morphological features of the reproductive systems and the measurements of developing stages, aid in identifying this species in tissue sections (Fig. 3).

SURVEYS OF THE INTERMEDIATE AND PARATENIC HOSTS

Various molluscan species have been known to serve as intermediate hosts for *A. cantonensis*. Among 56 species listed by Alicata and Jindrak (1970) as intermediate host

Table 4

Morphometric data of the adults of *Angiostrongylus cantonensis* from various districts (mm)

Author Locality	Present author Okinawa (Ryukyu)	Nishimura *et al.* (1964) Iriomote (Ryukyu)	Yamashita *et al.* (1978) Yoron (Amami)	Hori and Kusui (1972) Tokyo (Honshu)	Ohbayashi and Orihara (1968) Sapporo (Hokkaido)	Present author Taiwan	Ohbayashi and Orihara (1968) Hawaii
Male							
No. examined	12	2	30	10	2	12	4
Body length	20.1 (17.4-23.6)	16-18	18.95	21.31 (19.90-22.37)	22.6, 23.2	20.4 (19.3-21.6)	22.5 (21.7-23.4)
Body width	0.314 (0.282-0.348)	0.25	0.30	0.3 (0.29-0.35)	0.41, 0.41	0.349 (0.319-0.399)	0.36 (0.35-0.38)
Esophagus length	0.315 (0.289-0.333)	0.28-0.30	0.31	0.3 (0.25-0.35)	0.288, 0.304	0.294 (0.274-0.329)	0.33 (0.30-0.34)
Length from cephalic apex to nerve ring	0.227 (0.207-0.242)					0.214 (0.185-0.252)	
Length from cephalic apex to excretory pore	0.414 (0.370-0.455)					0.424 (0.385-0.466)	
Length of spicule	1.23 (1.13-1.34)	1.15	1.14	1.22 (1.12-1.27)	1.060, 1.198	1.22 (1.03-1.32)	1.18 (1.07-1.28)

Table 4 (Continued)

Author Locality	Present author Okinawa (Ryukyu)	Nishimura *et al.* (1964) Iriomote (Ryukyu)	Yamashita *et al.* (1978) Yoron (Amami)	Hori and Kusui (1972) Tokyo (Honshu)	Ohbayashi and Orihara (1968) Sapporo (Hokkaido)	Present author Taiwan	Ohbayashi and Orihara (1968) Hawaii
Female							
No. examined	12	6	30	10	1	12	4
Body length	28.5 (25.2-36.4)	17-18	32.24	29.89 (26.83-40.36)	32.5	29.2 (24.4-33.8)	31.15 (28.3-33.2)
Body width	0.474 (0.442-0.522)	0.3	0.51	0.43 (0.4-0.5)	0.66	0.467 (0.406-0.537)	0.47 (0.45-0.51)
Esophagus length	0.336 (0.315-0.359)		0.33	0.36 (0.3-0.4)	0.314	0.306 (0.244-0.359)	0.37 (0.34-0.38)
Length from cephalic apex to nerve ring	0.245 (0.222-0.266)					0.221 (0.167-0.274)	
Length from cephalic apex to excretory pore	0.412 (0.370-0.470)					0.417 (0.392-0.448)	
Length from tail tip to vulva	0.249 (0.196-0.318)	0.25	0.26	0.19 (0.16-0.23)	0.200	0.222 (0.189-0.259)	0.23 (0.21-0.25)
Length from tail tip to anus	0.058 (0.048-0.067)	0.06	0.05	0.05 (0.03-0.06)	0.052	0.058 (0.052-0.074)	0.06 (0.06-0.06)

Figure 2. Each stage of *Angiostrongylus cantonensis* (Yoron strain) one grade=10μ a. Adult male (left) and female (right) from the rat-lung collected on Yoron, Amami Islands. b. Posterior end of adult male (left) and female (right). c. First-stage larva from feces of the rat experimentally infected. d. Second-stage larva from the giant African snail collected on Yoron. e. Third-stage larva (moulted). f. Third-stage larvae encysted in the muscle. g. Male (left) and female (right) fouth-stage larvae from the brain of rat 10 days after experimental infection.

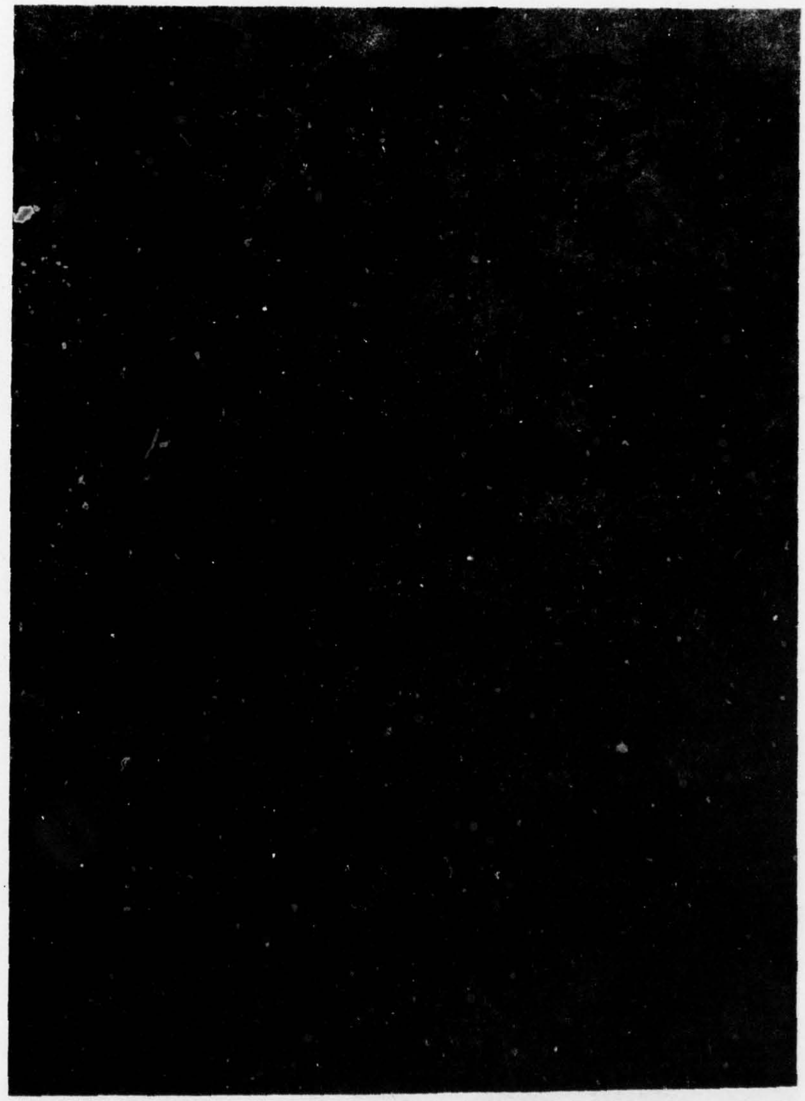

Figure 3. Cross sections through various levels of the young adult *Angiostrongylus cantonensis* (by courtesy of Dr. Y. Ohmori) a-d. ♀ worm in brain of rat 24 days after infection. e-i. ♂ worm in ditto. a. Anterior part of the ovaries. b. Level of oviducts. c. Level of the sphincter muscle of oviduct-uterus junction. d. Level of the uteri. e. Esophageal level. f. Level of the testis. g. and h. Posterior parts of the body. i. Bursa copulatorix. Bu: bursa copulatorix, CM: copulatory muscle, Co: coelomocyte, DC: dorsal chord, E: esophagus Ej: ejaculatory duct, I: intestine, LC: lateral chord, MC: median chord, O: ovary, Od: oviduct, Ra: rachis, SmC: submedian chord, Spc: spicule, Sph: sphincter, T: testis, U: uterus, Va: vagina, VC: ventral chord.

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STUDIES ON ANGIOSTRONGYLIASIS IN EASTERN ASIA AND AUSTRALIA. (U)

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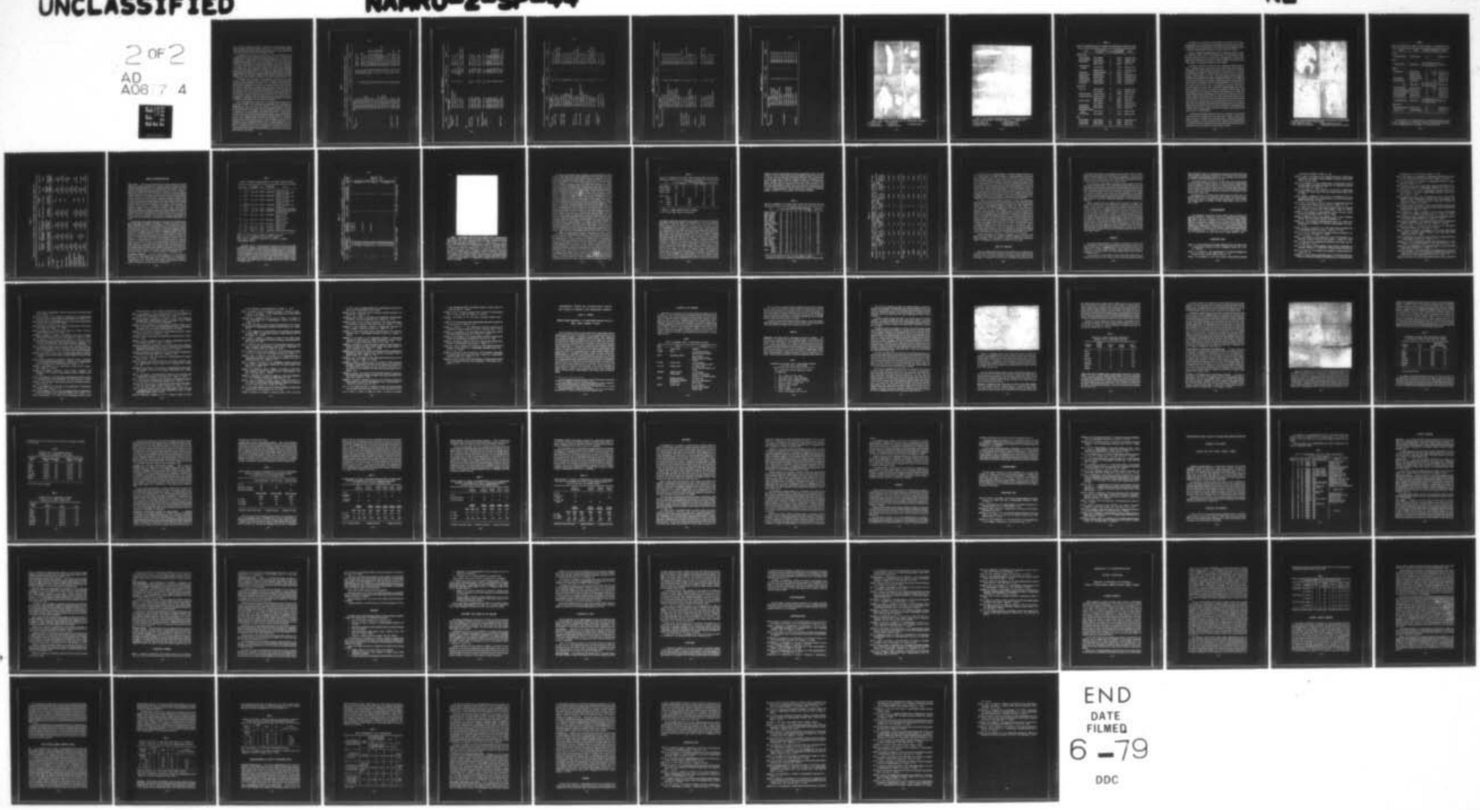

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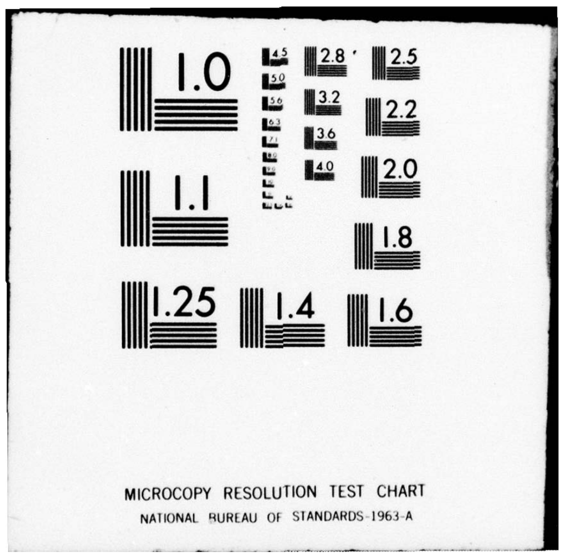
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MICROCOPY RESOLUTION TEST CHART
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under natural and experimental conditions, 6(allied) species *Cipangopaludina chinensis, Sinotaia quadrata, Deroceras reticulatum, Limax flavus, L. maximus* and *Indoplanorbis exustus* are distributed throughout Japan. The last species escaped from tanks rearing tropical fishes and is now seen in the natural environments in Japan.

The molluscan species which have been known to serve as intermediate hosts for *A. cantonensis* under natural conditions in Japan are shown in Table 5. In Okinawa and southward there are many terrestrial snails and slugs with high rates of infection (Fig. 4). *D. varians* and *L. marginatus* (Fig. 5 a, b) were found infected with the parasite in the Shinagawa and Harumi areas, Tokyo, where the natural infection of rats has also been observed. *A. fulica* was found infected in Chichijima and Hahajima, the West Pacific Ocean (Hori *et al.*, 1973b, 1974). Recently, the author found *D. varians* infected with *A. cantonensis* in Yokosuka south of Tokyo. *A. fulica* and *L. alte* (Fig. 4 a, b) which are widely distributed in the Ryukyu and Amami Islands have frequently been found infected on Okinawa, Ishigaki and elsewhere (Nishimura *et al.*, 1965; Nishimura, 1966b). Intermill *et al.* (1972) found *Bradybaena circulus, Fruticicola despecta, Satsuma mercatoria* (Fig. 4c, d, e), *Vaginulus plebeius, D. laeve* and *Philomycus bilineatus* naturally infected on Okinawa, and Asato and Kishimoto (1976a) confirmed the infection in the former 4 species as well as *L. alte*.

The Okinawa Prefectural Institute of Public Health has been surveying for naturally infected *A. fulica* in the Ryukyu Islands since 1971. The results obtained through these surveys are as follows (Kishimoto and Asato, 1974): In Okinawa, the highest infection rate was found in the middle part (57.6%), next in the southern part (28.5%) and the northern part (23.4%), corresponding to the density of the snail in these 3 cases. This snail is not seen on Izena, the islet located northwest of Okinawa, although the *A. cantonensis* infection rate in rats was found to be 41.9%. Molluscan intermediate hosts other than *A. fulica* may be responsible for the murine infection. On the other hand, *A. fulica* inhabits Iheya, the islet located north of Izena, and it has been found infected with *A. cantonensis*, but at a low rate (0.8%). In addition to Okinawa, the infection rate of *A. fulica* was high on Miyako and Ishigaki, two main islands south of the Okinawa Island, and the parasite was also found on Kitadaito and Minamidaito, the remote islets east of Okinawa.

In the Amami Islands (Fig. 1) located north of the Ryukyu Islands, the giant African snail is known to inhabit Yoron, Tokunoshima and Amamioshima, but not Okinoerabu and Kikaigashima. This snail is not native to these islands but was imported from Taiwan and Oceania probably between 1935 to 1940, and since has propagated markedly. In 1937-1938, the snail was imported to Tokunoshima in the Amami Islands, and seems to have extended its distribution to the other islands by various routes through 1947. *A. cantonensis* has not been recovered from the snail on the Amami Islands. Recently, in Setouch, Amamioshima, one out of 40 *A. fulica* was found infected with the third-stage larvae of *A. cantonensis* (Kurihara, 1976). Successively, Ishida *et al.* (1977) reported *A. fulica* to be infected with the third-stage larvae in Yoron and Tokunoshima, as well as in Makurazaki and Ijuin of the southernmost Prefecture (Kagoshima), Kyushu, where *F. despecta sieboldiana* was found infected. As mentioned above, the adult worm was found in *R. norvegicus* along with the larvae in *A. fulica* and *B. circulus* in Yoron (Yamashita *et al.*, 1978). These facts show that the parasite has already extended its distribution along the northern islands of the Southwest Islands further to Japan Proper.

Table 5

Surveys of natural infections of mollusks with *Angiostrongylus cantonensis* in Japan

Species	Locality	No. examined	No. Infected (%)	Reference
Snails	**Positive findings**			
Achatina fulica	Okinawa (Ryukyu)		positive	Nishimura *et al.*, 1965
	Okinawa (Ryukyu)	10	positive	Nishimura, 1966b
	Miyako (Ryukyu)	10	positive	
	Okinawa (Ryukyu)	35	20 (57.1)	Yanagisawa *et al.*, 1969a
	Okinawa (Ryukyu)	1090	positive	Intermill *et al.*, 1972
	Chichijima (Ogasawara)	397	88 (22.2)	Hori *et al.*, 1973b
	Hahajima (Ogasawara)	171	11 (6.4)	Hori *et al.*, 1974
	Okinawa (Ryukyu)	1915	855 (44.6)	Kishimoto and Asato, 1974
	Ishigaki (Ryukyu)	103	60 (58.3)	Kishimoto and Asato, 1974
	Kitadaito (Ryukyu)	20	1 (5.0)	Kishimoto and Asato, 1974
	Iheya (Ryukyu)	120	1 (0.8)	Kishimoto and Asato, 1974
	Kume (Ryukyu)	193	61 (31.6)	Asato and Kishimoto, 1976a
	Minamidaito (Ryukyu)	201	1 (0.5)	Asato and Kishimoto, 1976a
	Miyako (Ryukyu)	130	71 (54.6)	Asato and Kishimoto, 1976a
	Amamioshima (Amami)	40	1 (2.5)	Kurihara (preliminary report), 1976
	Amamioshima (Amami)	50	1 (2.0)	Ishida *et al.*, 1977
	Tokunoshima (Amami)	209	8 (3.8)	Ishida *et al.*, 1977
	Yoron (Amami)	62	32 (51.6)	Ishida *et al.*, 1977
	Yoron (Amami)	528	30 (5.7)	Yamashita *et al.*, 1978
Bradybaena circulus	Okinawa (Ryukyu)	2276	positive	Intermill *et al.*, 1972
	Okinawa (Ryukyu)	435	3 (0.7)	Asato and Kishimoto, 1976a
	Yoron (Amami)	90	6 (6.7)	Yamashita *et al.*, 1978
Fruticicola despecta	Okinawa (Ryukyu)	2199	positive	Intermill *et al.*, 1972
	Okinawa (Ryukyu)	303	3 (1.0)	Asato and Kishimoto, 1976a

Table 5 (Continued 1)

Species	Locality	No. examined	No. infected (%)	Reference
Snails	**Positive findings**			
Fruticicola despecta sieboldiana	Makurazaki, Kagoshima (Honshu)	58	positive	Ishida *et al.*, 1977
	Ijuin, Kagoshima (Honshu)	21	positive	Ishida *et al.*, 1977
Satsuma mercatoria	Okinawa (Ryukyu)	351	positive for 1st-stage larvae	Intermill *et al.*, 1972
	Okinawa (Ryukyu)	255	35 (13.7)	Asato and Kishimoto, 1976a
	Izena (Ryukyu)	5	1 (20.0)	Asato and Kishimoto, 1976a
Slugs				
Deroceras laeve	Okinawa (Ryukyu)	4992	positive	Intermill *et al.*, 1972
Deroceras varians	Shinagawa (Honshu)	139	52 (37.4)	Hori *et al.*, 1973a
	Harumi (Honshu)	7	2 (28.6)	Hori *et al.*, 1973a
	Yokosuka (Honshu)	73	5 (6.9)	Otsuru, 1978
Limax marginatus	Shinagawa (Honshu)	165	38 (23.5)	Hori *et al.*, 1973a
	Harumi (Honshu)	130	52 (40.0)	Hori *et al.*, 1973a
Philomycus (Meghimatum) bilineatus	Okinawa (Ryukyu)	1596	positive	Intermill *et al.*, 1972
Laevicaulis alte	Miyako (Ryukyu)	10	positive	Nishimura, 1966b
	Ishigaki (Ryukyu)	10	positive	Nishimura, 1966b
	Iriomote (Ryukyu)	10	positive	Nishimura, 1966b
	Okinawa (Ryukyu)	66	24 (36.4)	Asato and Kishimoto, 1976a
	Miyako (Ryukyu)	44	24 (54.5)	Asato and Kishimoto, 1976a
	Iriomote (Ryukyu)	85	11 (12.9)	Asato and Kishimoto, 1976a
	Yonaguni (Ryukyu)	105	3 (2.9)	Asato and Kishimoto, 1976a
	Izena (Ryukyu)	60	19 (31.7)	Asato and Kishimoto, 1976a
Vaginulus plebeius	Okinawa (Ryukyu)	353	positive	Intermill *et al.*, 1972
	Okinawa (Ryukyu)	22	2 (9.1)	Asato and Kishimoto, 1976a

Table 5 (Continued 2)

Species	Locality	No. examined	Result	Reference
Snails	**Negative findings**			
Achatina fulica	Amamioshima (Amami)	600	–	Kamiya *et al.*, 1968
Bradybaena circulus	Okinawa (Ryukyu)	34	–	Yanagisawa *et al.*, 1969a
	Minnajima (Ryukyu)	6	–	Asato and Kishimoto, 1976a
	Izena (Ryukyu)	13	–	Asato and Kishimoto, 1976a
Bradybaena oceania	Hahajima (Ogasawara)	30	–	Hori *et al.*, 1974
Bradybaena similaris	Okinoerabu (Amami)	193	–	Ishida *et al.*, 1977
	Amamioshima (Amami)	225	–	Ishida *et al.*, 1977
	Kikaigashima (Amami)	54	–	Ishida *et al.*, 1977
	Tanegashima (Amami)	65	–	Ishida *et al.*, 1977
	Kushikino, Kagoshima (Kyushu)	46	–	Ishida *et al.*, 1977
Cliton sower	Okinawa (Ryukyu)	99	–	Intermill *et al.*, 1972
Cyclophorus turgidus	Okinawa (Ryukyu)	10	–	Yanagisawa *et al.*, 1969a
	Okinawa (Ryukyu)	163	–	Asato and Kishimoto, 1976a
Cyclophorus herklotsi	Shimokoshikijima, Kagoshima (Kyushu)	12	–	Ishida *et al.*, 1977
	Furue, Kagoshima (Kyushu)	11	–	Ishida *et al.*, 1977
	Kagoshima (Kyushu)	19	–	Ishida *et al.*, 1977
Euglandina rosea	Chichijima (Ogasawara)	4	–	Hori *et al.*, 1974
Fruticicola despecta	Okinawa (Ryukyu)	34	–	Yanagisawa *et al.*, 1969a
	Miyako (Ryukyu)	41	–	Asato and Kishimoto, 1976a
	Izena (Ryukyu)	53	–	Asato and Kishimoto, 1976a
	Yoron (Amami)	17	–	Yamashita *et al.*, 1978
Fruticicola despecta sieboldiana	Okinoerabu (Amami)	53	–	Ishida *et al.*, 1977
	Amamioshima (Amami)	13	–	Ishida *et al.*, 1977
	Kikaigashima (Amami)	57	–	Ishida *et al.*, 1977

Table 5 (Continued 3)

Species	Locality	No. examined	Result	Reference
Snails	**Negative findings**			
Fruticicola despecta sieboldiana	Nakanoshima (Amami)	3	–	Ishida *et al.*, 1977
	Yakushima (Amami)	76	–	Ishida *et al.*, 1977
	Tanegashima (Amami)	259	–	Ishida *et al.*, 1977
	Shimokoshikijima, Kagoshima (Kyushu)	80	–	Ishida *et al.*, 1977
	Kokubu, Kagoshima (Kyushu)	32	–	Ishida *et al.*, 1977
	Kanoya, Kagoshima (Kyushu)	60	–	Ishida *et al.*, 1977
	Furue, Kagoshima (Kyushu)	3	–	Ishida *et al.*, 1977
	Yamakawa, Kagoshima (Kyushu)	150	–	Ishida *et al.*, 1977
	Kushikino, Kagoshima (Kyushu)	208	–	Ishida *et al.*, 1977
	Kagoshima (Kyushu)	12	–	Ishida *et al.*, 1977
Leptopoma perlucidum	Okinawa (Ryukyu)	2	–	Yanagisawa *et al.*, 1969a
Satsuma mercatoria	Yoron (Amami)	4	–	Otsuru, 1978
Assiminea luta japonica	Okinawa (Ryukyu)	100	–	Asato and Kishimoto, 1976a
Euhadra herklotsi	Shibushi, Kagoshima (Kyushu)	48	–	Ishida *et al.*, 1977
	Kushikino, Kagoshima (Kyushu)	3	–	Ishida *et al.*, 1977
	Kagoshima (Kyushu)	3	–	Ishida *et al.*, 1977
Pupinella rufa	Kagoshima (Kyushu)	293	–	Ishida *et al.*, 1977
Slugs				
Incilaria fruhstorferi	Okinawa (Ryukyu)	16	–	Asato and Kishimoto, 1976a
Incilaria bilineata	Okinawa (Ryukyu)	24	–	Asato and Kishimoto, 1976a
	Yokosuka (Honshu)	45	–	Otsuru, 1978
	Okinoerabu (Amami)	3	–	Ishida *et al.*, 1977
	Yakushima (Amami)	54	–	Ishida *et al.*, 1977
	Chosa, Kagoshima (Kyushu)	53	–	Ishida *et al.*, 1977

Table 5 (Continued 4)

Species	Locality	No. examined	Result	Reference
Slugs	**Negative findings**			
Incilaria bilineata	Kokubu, Kagoshima (Kyushu)	61	–	Ishida *et al.*, 1977
	Shibushi, Kagoshima (Kyushu)	72	–	Ishida *et al.*, 1977
	Furue, Kagoshima (Kyushu)	47	–	Ishida *et al.*, 1977
	Ijuin, Kagoshima (Kyushu)	26	–	Ishida *et al.*, 1977
	Kushikino, Kagoshima (Kyushu)	89	–	Ishida *et al.*, 1977
	Kagoshima (Kyushu)	59	–	Ishida *et al.*, 1977
Limax marginatus	Yokosuka (Honshu)	47	–	Otsuru *et al.*, 1978
	Sakurajima, Kagoshima (Kyushu)	110	–	Ishida *et al.*, 1977
	Makurazaki, Kagoshima (Kyushu)	51	–	Ishida *et al.*, 1977
	Yamakawa, Kagoshima (Kyushu)	59	–	Ishida *et al.*, 1977
	Kagoshima (Kyushu)	12	–	Ishida *et al.*, 1977
Limax flavus	Tanegashima (Amami)	33	–	Ishida *et al.*, 1977

Figure 4. Some molluscan intermediate hosts for ***Angiostrongylus cantonensis*** in the Ryukyu Islands. one grade=1 mm

a. *Achatina fulica* b. *Laevicaulis alte* c. *Bradybaena circulus*

d. *Fruticicola despecta* e. *Satsuma mercatoria*

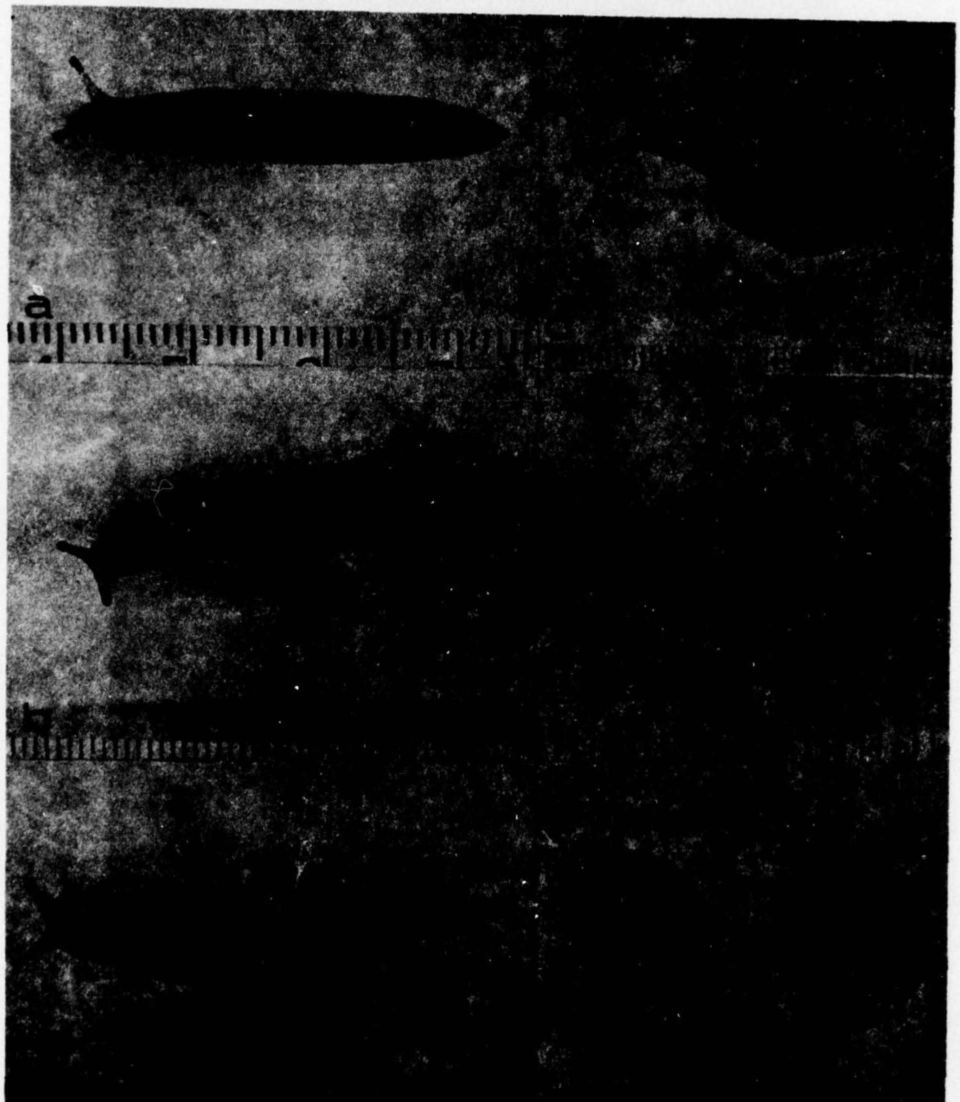

Figure 5. Some molluscan intermediate hosts for *Angiostrongylus cantonensis* in Honshu (N: natural infection, E: experimental infection). one grade=1 mm

a. *Deroceras varians* (N)
b. *Limax marginatus* (N)
c. *Incilaria bilineata* (E)
d. *Euhadra quaesita* (E)
ε. *Bakerlymnaea viridis* (E)
f. *Cipangopaludina* sp. (E)

Table 6

Results of experimental infections of mollusks with *Angiostrongylus cantonensis* in Japan

Species	Locality of mollusks examined	No. examined	No. infected (%) (third-stage larvae)	Reference
Snails (terrestrial)				
Allopeas kyotoensis	Tokyo (Honshu)	2	2 (100)	Yanagisawa, 1967b
Euhadra peliomphala	Tokyo (Honshu)	3	2 (66.7)	Yanagisawa, 1967b
Euhadra quaesita	Saitama (Honshu)	8	6 (75.0)	Yangisawa, 1967b
	Niigata (Honshu)	50	50 (100)	Otsuru, 1978
Fruticicola despecta sieboldiana	Tokyo (Honshu)	3	3 (100)	Yanagisawa, 1967b
	Ishigaki (Ryukyu)	6	2 (33.3)	Hori and Kano, 1974
Achatina fulica	Chichijuma (Ogasawara)	24	21 (87.1)	Hori, 1973
Bradybaena oceania	Ogasawara Islands	33	33 (100)	Hori and Kano, 1974
Euhadra hickonis	Okayama (Honshu)	6	3 (50.0)	Hori and Kano, 1974
Zonitoides arboreus	Tokyo (Honshu)	9	9 (100)	Yanagisawa, 1967b
Fruticicola despecta	Ishigaki (Ryukyu)	6	2 (33.3)	Hori and Kano, 1974
Succinea lauta	Saitama (Honshu)	13	9 (69.2)	Hori *et al.*, 1976
Snails (aquatic)				
Physa acuta	Saitama (Honshu)	15	1 (6.7)	Yanagisawa, 1967b
	Kyoto (Honshu)		positive	Arizono *et al.*, 1976
Indoplanorbis exustus	Kanagawa (Honshu)	22	9 (40.9)	Hori *et al.*, 1976
Semisulcospira libertina	Kanagawa (Honshu)	33	1 (3.0)	Hori *et al.*, 1976
	Niigata (Honshu)	25	positive	Otsuru, 1978
Bakerlymnea viridis	Niigata (Honshu)	50	positive	Otsuru, 1978
	Kyoto (Honshu)		positive	Arizono *et al.*, 1976
Lymnaea japonica	Kyoto (Honshu)		positive	Arizono *et al.*, 1976
Cipangopaludina sp.	Niigata (Honshu)	15	positive	Otsuru, 1978
Cipangopaludina chinensis malleata	Kyoto (Honshu)	11	1 (9.1)	Shiota *et al.*, 1977
Gyraulus chinensis	Kyoto (Honshu)	16	6 (37.5)	Shiota *et al.*, 1977
Slugs				
Limax marginatus	Tokyo (Honshu)	15	15 (100)	Hori and Kano, 1974
Deroceras varians	Niigata (Honshu)	200	positive	Otsuru, 1978
Incilaria bilineata	Niigata (Honshu)	150	positive	Otsuru, 1978

The finding of *A. cantonensis* in rats and mollusks in the harborside areas of Tokyo, strongly suggests that the parasite may have been imported by these hosts and the life cycle is established in the area. The discovery of the parasite from rats in Hokkaido is similar to the above findings, although it appears to be too far north (43°N. latitude).

Results of experimental molluscan infection with *A. cantonensis* carried out in Japan are summarized in Table 6. Most of the examined species (10 terrestrial snails, 6 aquatic snails and 3 slugs) were known to be infected with *A. cantonensis* (Fig. 5 c-f). Such results suggest that the distribution of the parasite is capable of extending to more areas of Japan Proper because of the wide-spread distribution of the mollusks.

The results of the survey of paratenic hosts for *A. cantonensis* in Japan are shown in Table 7. Third-stage larvae have been found naturally in *Bufo asiaticus* (Fig. 6a) of Miyako and Kitadaito, and in *Rana catesbeiana* (Fig. 6b) of Kume, the islet west to Okinawa. *R. limnocharis* and *Rhacophorus leucomystax* (Fig. 6c, d) were also found to be naturally infected on Okinawa (Asato *et al.*, 1978). A hairy crab, *Eriocheir japonicus* of Okinawa, 2 freshwater prawns, *Macrobrachium longipes* of Okinawa and *Palaemon* sp. of the Ogasawara Islands were also examined, but were negative. Experimental infections with the third-stage larvae were done with *Cambarus clarkii* and *Cynops pyrrhogaster* by the author, but the results showed that both species were of little importance as paratenic hosts. Kinjo *et al.* (1975a) reported 2 human cases of eosinophilic meningoencephalitis who were infected by taking the raw liver of *B. asiaticus* as prescribed in traditional Chinese medicine. Subsequently other *B. asiaticus* from Miyako were examined and 35 (42.2%) of 83 found naturally harboring infective-stage larvae. The larvae were recovered from the toad stomach, intestinal wall, mesentery, liver and muscle. The maximum number of larvae recovered from one toad was 3,256. The larvae were still alive after a lapse of more than 20 days. In the survey of natural infection of frogs, *Hyla aurea* was found to harbor infective-stage larvae in New Caledonia by Ash (1968). However, human infection with *A. cantonensis* through the toad has never been reported. Finding of the parasite in *R. catesbeiana* has some importance since this frog is eaten in some areas in the Ryukyu Islands and such eating habits may lead to angiostrongyliasis.

The molluscan intermediate and paratenic hosts for *A. cantonensis* on Taiwan Proper determined by the author's Department are presented in Table 3. Similar to reports made by others *A. fulica* and *C. chinensis* were found infected with *A. cantonensis*, but *Semisulcospira* sp. was negative. In a survey of paratenic hosts, *R. tigrina* (3.2%), *R. plancyi* (21.4%), *R. latouchi* (21.4%) and *B. melanostrictus* (8.3%) were found naturally infected with third-stage larvae. The former 2 species are commonly eaten on Taiwan.

Figure 6. Toad and frogs naturally infected with the third-stage larvae of *Angiostrongylus cantonensis* (paratenic host) in the Ryukyu Islands.

a. *Bufo asiaticus* from Miyako
b. *Rana catesbeiana* from Kume
c. *Rana limnocharis* from Okinawa
d. *Rhacophorus leucomystax* from Okinawa

Table 7

Surveys of paratenic hosts in Japan (N: natural infection, E: experimental infection)

Species	Locality	No. examined	No. infected (%)	Reference
Land crab				
Eriocheir japonicus (N)	Okinawa (Ryukyu)	44	–	Intermill *et al.*, 1972
Crayfish				
Cambarus clarkii (E)	Niigata (Honshu)	The third-stage larvae were alive up to 7 days after infection		Otsuru, 1978
Amphibia				
Bufo asiaticus (N)	Miyako (Ryukyu)	83	35 (42.2)	Asato *et al.*, 1978
	Kitadaito (Ryukyu)	10	1 (10.0)	Asato *et al.*, 1978
	Minamidaito (Ryukyu)	14	–	Asato *et al.*, 1978
Bufo marinus (N)	Minamidaito (Ryukyu)	26	–	Asato *et al.*, 1978
Rana catesbeiana (N)	Kume (Ryukyu)	44	7 (15.9)	Asato *et al.*, 1978
(E)	Niigata (Honshu)	The third-stage larvae were alive over 70 days after infection		Asato *et al.*, 1978
Rana limnocharis (N)	Okinawa (Ryukyu)	8	1 (12.5)	Asato *et al.*, 1978
Rhacophorus leucomystax (N)	Okinawa (Ryukyu)	15	1 (6.7)	Asato *et al.*, 1978
Rhacophorus japonicus (N)	Okinawa (Ryukyu)	8	–	Asato *et al.*, 1978
Rhacophorus viridis (N)	Okinawa (Ryukyu)	5	–	Asato *et al.*, 1978
Microhyla ornata (N)	Okinawa (Ryukyu)	1	–	Asato *et al.*, 1978
Cynops pyrrhogaster (E)	Niigata (Honshu)	The third-stage larvae were alive up to 4 days after infection		Otsuru , 1978
Freshwater prawn				
Macrobrachium longipes (N)	Okinawa (Ryukyu)	129	–	Intermill *et al.*, 1972
Palaemon sp. (N)	Chichijima (Ogasawara)	180	–	Hori *et al.*, 1974

The measurements of the third-stage larvae of *A. cantonensis* from Japan are shown in Table 8, comparing them with larvae from other districts. The morphometric data, along with the morphologic features, are comparable to each other (Fig. 2e, f).

Table 8

Morphometric data of the third-stage larvae of *Angiostrongylus cantonensis* from various districts (mm)

Author	Present author	Hori *et al.*, 1973a	Ishida *et al.*, 1977	Ishida *et al.*, 1977	Yamashita *et al.*, 1978	Present author	Alicata, 1963
Locality	Yokosuka port (Honshu)	Harborside areas of Tokyo (Honshu)	Makurazaki, Kagoshima (Kyushu)	Tokunoshima (Amami)	Yoron (Amami)	Taiwan	Hawaii
Intermediate host	*Deroceras varians* (natural infection)	*Limax marginatus* (natural infection)	*Fruticicola despecta sieboldiana* (natural infection)	*Achatina fulica* (natural infection)	*Achatina fulica* (natural infection)	*Biomphalaria glabrata* (experimental infection)	*Deroceras laeve* (experimental infection)
No. examined	40	20	10	10	20	20	10
Body length	0.46 (0.42-0.51)	0.46 (0.44-0.49)	0.450 (0.421-0.483)	0.487 (0.465-0.525)	0.50	0.40 (0.36-0.44)	0.48 (0.46-0.51)
Body width	0.023 (0.021-0.029)	0.02 (0.02-0.03)	0.021 (0.019-0.022)	0.026 (0.024-0.029)	0.028	0.022 (0.019-0.030)	0.026 (0.026-0.026)
Esophagus length	0.17 (0.15-0.19)	0.18 (0.16-0.19)	0.189 (0.174-0.205)	0.194 (0.173-0.221)	0.171	0.15 (0.13-0.18)	0.18 (0.17-0.20)
Length from cephalic apex to nerve ring	0.074 (0.069-0.081)	0.09 (0.07-0.09)				0.067 (0.063-0.074)	0.07
Length from cephalic apex to excretory pore	0.089 (0.081-0.096)		0.083 (0.078-0.090)	0.083 (0.071-0.089)	0.085	0.079 (0.070-0.093)	0.09 (0.08-0.09)
Length from cephalic apex to genital primordium	0.30 (0.26-0.33)	0.29 (0.27-0.31)	0.276 (0.258-0.300)	0.292 (0.269-0.313)	0.300	0.25 (0.20-0.30)	(0.34-0.38)
Length from tail tip to anus	0.038 (0.030-0.044)	0.04	0.040 (0.039-0.045)	0.038 (0.032-0.044)	0.039	0.031 (0.024-0.039)	0.041 (0.037-0.044)

HUMAN ANGIOSTRONGYLIASIS

Cases in Japan. Since 1964, *A. cantonensis* has been recovered frequently from various molluscan intermediate and definitive hosts collected in the Ryukyu Islands. On Taiwan, eosinophilic meningoencephalitis due to *A. cantonensis* had been recognized first in 1944, and since 1964 numerous cases have been reported. Due to the proximity of Taiwan to Okinawa the possibility that human infection with *A. cantonensis* may occur in the Ryukyu Islands became of some concern. Up to the present time, 12 cases of eosinophilic meningoencephalitis, probably due to this parasite, have been reported in the islands, and are summarized in Table 9. Simpson *et al.* (1970) reported 3 cases and Yonamine and Ashimine (1972) added another case. They indicated that these cases might be caused by eating or handling *A. fulica.* Yonamine (personal communication) treated 2 cases on Miyako probably acquired from swallowing slugs used in Chinese medicine in 1970 and 1974. Nakamoto *et al.* (1974) reported a similar case caused by swallowing a slug in Miyako, and on ophthalmoscopic examination a young worm of *A. cantonensis* was visualized. In 1975, 2 other cases were reported from Miyako by Kinjo *et al.*, and liver of a toad, *B. asiaticus,* which was freshly swallowed as a medicine by each patient, was the suspected source of infection. Subsequently, 2 other cases were added by Nakamoto (1976) and Ashimine (1976) in the same year. Recently, 2 larvae of *A. cantonensis,* a male and female, 5.44 and 2.75 mm in length and 107 and 70 μ in width, respectively, were recovered from cerebrospinal fluid (CSF) of case 12 (Table 9); this is the first report of recovering larvae from CSF in Japan (Asato *et al.*, 1977).

In Japan Proper, human angiostrongyliasis has not been reported, but positive immunologic findings suggest the possibility of the disease. In Yokosuka, one patient with marked signs of meningitis had a positive skin test showing 10 x 10 mm wheal and 30 x 25 mm erythema and a weak positive in both the Ouchterlony reaction and in the indirect hemagglutination (IHA) test; serum titer of 1:64 (Otsuru *et al.*, 1976). Unfortunately, the CSF was not examined until 12 months after onset of disease and consequently eosinophilic pleocytosis important for the diagnosis, was not be observed. In Kagoshima, Tomihira *et al.* (1977) reported a case of meningoencephalitis in which an immunoelectrophoretic precipitin band, specific for *A. cantonensis,* was demonstrated between the serum and extract of *A. cantonensis.* Kojima *et al.* (1978) reported eosinophilic meningoencephalitis in Shizuoka which was considered to be angiostrongyliasis not only from the clinical symptoms and eosinophilic pleocytosis but also from the results of immunological testing. The skin test was strongly positive showing 25 x 18 mm wheal and the complement fixation test indicated a high titer. Positive results were also obtained both in the Ouchterlony reaction and in immunoelectrophoresis. Medicinal slugs were frequently orally administered to the patient over a 4 year period. Dohy and Tsuji (1977) reported 2 cases in which the patients showed peripheral eosinophilia and positive serologic reactions to *A. cantonensis* antigen in the gel-diffusion test.

Immunological observations. The recent 7 patients (cases 5, 7, 8, 9, 10, 11 and 12 in Table 9) in the Ryukyu Islands were examined immunologically in the author's Department (Sato *et al.*, 1977), and serologic surveys were also carried out on inhabitants in 5 islands (Otsuru *et al.*, 1977b). The results obtained by immunological examinations together with remarks on the field survey are described below.

Table 9

Cases of eosinophilic meningoencephalitis in the Ryukyu Islands, 1969-1977

Case No.	Age, Sex	Date of admission to hospital	Locality	Suspected source of infection	Reference
1	24, ♂	11 Apr., 1969	Okinawa	*Achatina fulica*	Simpson *et al.*, 1970 Yonamine and Ashimine, 1972
2	58, ♀	3 Jul., 1969	Okinawa	*Achatina fulica*	Simpson *et al.*, 1970 Yonamine and Ashimine, 1972
3	68, ♂	9 Sep., 1969	Okinawa	*Achatina fulica*	Simpson *et al.*, 1970 Yonamine and Ashimine, 1972
4	1 year & 8 months, ♂	26 May, 1970	Okinawa	*Achatina fulica*	Ashimine *et al.*, 1970 Yonamine and Ashimine, 1972 Ashimine, 1976
5*	47, ♀	Oct., 1970	Miyako	*Deroceras laeve*	Yonamine (personal communication)
6	43, ♂	Apr., 1974	Miyako	*Laevicaulis alte*	Yonamine (personal communication)
7*	34, ♀	11 May, 1974	Miyako	*Laevicaulis alte*	Nakamoto *et al.*, 1974 Kinjo *et al.*, 1975b Nakamoto, 1976
8*	38, ♀	21 Feb., 1975	Miyako	*Bufo asiaticus*	Kinjo *et al.*, 1975a, b Nakamoto, 1976
9*	20, ♂	21 Feb., 1975	Miyako	*Bufo asiaticus*	Kinjo *et al.*, 1975a, b Nakamoto, 1976
10*	53, ♀	23 Oct., 1975	Okinawa	*Achatina fulica*	Nakamoto, 1976
11*	1, ♀	27 Dec., 1975	Okinawa	unknown	Ashimine, 1976
12*	49, ♂	14 Feb., 1977	Miyako	*Achatina fulica*	Hanada (personal communication)

Cases 1, 2, 3, 4, 10 and 12: eating or handling of the snail.
Cases 5, 6 and 7: swallowing of the slug as a medicine.
Cases 8 and 9: swallowing of fresh liver of the toad as a medicine.
*immunologically examined

Young adult worms of *A. cantonensis* have been found in the CSF only on rare cases and therefore, the diagnosis of angiostrongyliasis is usually based on clinical characteristics and results of immunological testing. The immunological diagnosis, however, has been applied only to a few cases. Sato *et al.* (1974) used immunoadsorbent columns for the preparation of a specific and highly responsive skin-test antigen. After dialysis against an excess of distilled water and lyophilization of the antigen preparation, sterilized physiological saline was added to make a protein concentration of 60 µg/ml. Fifteen minutes after intradermal injection with 0.02 ml of this antigen solution into the forearm, long and short diameters of wheals and erythemas were measured and the mean diameter of a wheal over 9 mm was defined as a positive reaction.

Table 10

Results of immunological reactions on seven suspected cases of angiostrongyliasis in the Ryukyu Islands.

Case No.	Time elapsed after the suspected infection	Skin reaction (wheal/erythema)	Ouchterlony reaction	No. of precipitin bands in immunoelectrophoresis	Reciprocal IHA titers
5	4 years & 5 months	12 x 11/30 x 25	–	–	<16
7	11 months	10 x 8/30 x 25	–	–	64
	40 days	11 x 11/20 x 20	+	6	4096
	72 days		+	6	8192
8	84 days		+	5	8192
	128 days		+	3	4096
	180 days		+	1	1024
	40 days	15 x 11/40 x 25	+	3	256
	72 days		+	3	512
9	84 days		+	3	512
	128 days		+	1	256
	180 days		+	1	256
	33 days	10 x 9/31 x 29	+	5	512 (256)
10	44 days		+	5	2048 (512)
	73 days		+	5	1024 (256)
11	20 days*		+	5	8192 (1024)
	10 days		–	–	16
	17 days		+	1	64 (<16)
	24 days		+	2	128 (32)
	32 days		+	4	512
	39 days		+	4	2048
12	46 days		+	5	2048
	53 days		+	4	1024
	67 days		+	3	1024
	81 days		+	3	512
	117 days		+	2	512
	150 days		+	2	256

*Time elapsed after the admission to hospital. ( ) IHA titers in the cerebrospinal fluids.

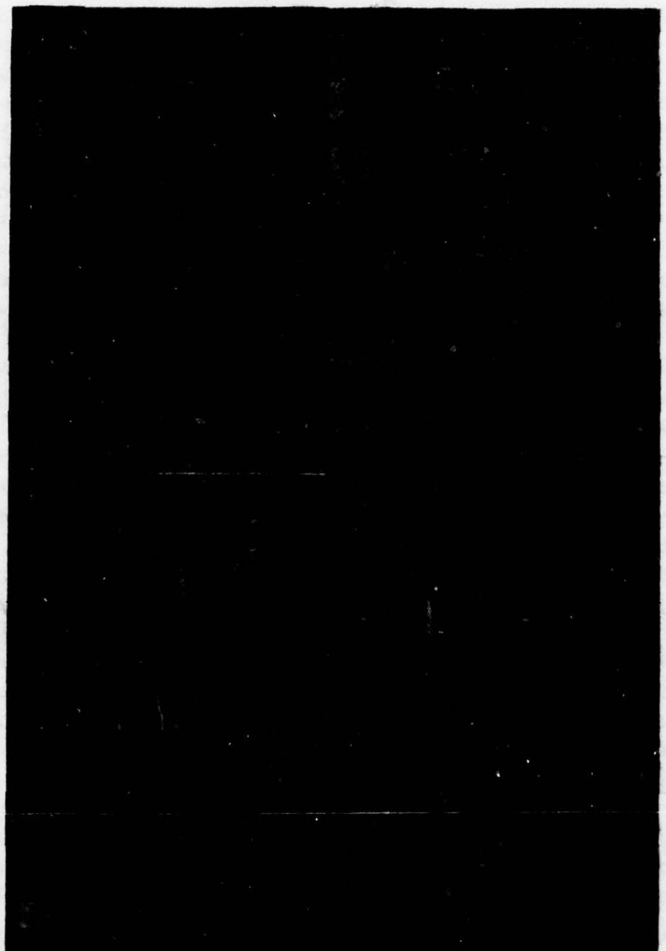

Figure 7. Immunological reactions on seven suspected cases of angiostrongyliasis in the Ryukyu Islands. a. Skin reaction on the forearm of Case 8, 15 minutes after intradermal injections with 0.02 ml of *A. cantonensis* antigen. The margins of wheals were outlined with a ball-point pen. Protein concentration of 1, 2, 3, 4, 5 and 6 were 3.5, 7, 14, 28, 56 and 112 μg/ml respectively, and physiological saline as a control. b. Ouchterlony reactions, of Cases 5, 7, 8, 9. 10 and 11. Ac: the whole worm extract of *A. cantonensis*, IRS: the infected rat serum, NHS: normal human serum, 5, 7, 8, 9. 10 and 11: the sera of Cases 5 at 4 years and 5 months, 7 at 11 months, 8 and 9 at 40 days, 10 at 33 days and 11 at 20 days after the suspected infection or admission to hospital, respectively. c. Ouchterlony reactions with the sera collected periodically from Case 12. Ac: the whole worm extract of *A. cantonensis.* Case 12: the sera of case 12 collected as indicated. d. Immunoelectrophoretic analysis of Case 12 showing a periodical change of precipitin bands. Wells were filled with the whole worm extract of *A. cantonensis* and troughs with the sera of Case 12 collected as indicated.

The results of immunological testing 7 suspected cases of angiostrongyliasis are shown in Table 10. Skin testing 5 cases showed positive reactions over 9 mm in mean size of wheal and over 20 mm in mean size of erythema. Figure 7a presents the results of a skin test with serially diluted antigen and physiological saline as a control in case 8. Protein concentration at each injected site was 3.5, 7, 14, 28, 56 and 112 μg/ml at No. 1, 2, 3, 4, 5 and 6, respectively. The intensity of reactions decreased with higher antigen dilutions and no reaction occurred at the site of physiological saline. The sera from the same 7 patients were tested in the IHA test with the antigen purified by DEAE-cellulose column and the immunoadsorbent column (Sato, 1975). The Ouchterlony gel-diffusion method and immunoelectrophresis were also used and the results are summarized in Table 10. IHA titers in the sera of case 5 and case 7, 4 years and 5 months and 11 months, respectively, after the suspected infection were low, < 1:16 and 1:64. Higher antibody titers ≥ 1:512 were obtained in cases 8, 9, 10, 11 and 12. In cases 8, 9, 10 and 11, titers of 1:256 and greater were detected in the first sera tested of each case and the titers remained high for over 6 months in cases 8 and 9. The antibody response in the early stage of infection was examined in case 12, in which a titer of 1:16 was found 10 days after suspected infection and the titer increased to 1:2048 during the next 29 days. Although antibodies were also detected in the CSF of cases 10, 11 and 12, they appeared later and the antibody levels were lower than those in the sera. In the Ouchterlony method, as shown in Figure 7b, c, the sera of cases 8, 9, 10, 11 and 12 showed positive reaction, but the sera of cases 5 and 7 were negative. In the immunoelectrophoretic analysis of these cases, 5 or 6 precipitin bands were demonstrated in the first sera from cases 8, 10 and 11, however, only 3 precipitin bands were seen in case 9. The numbers of such precipitin bands decreased with time after the suspected infection and all of the bands except one disappeared at 180 days in cases 8 and 9. Conversely, periodic examination of sera from case 12 showed a gradual increase of the precipitin bands (Fig. 7d); one precipitin band at 17 days increased to 5 at 46 days. In contradiction to the skin test, IHA antibodies and precipitating antibodies were produced within 1 month, but were not demonstrable 10 months or more after infection. This suggested, as demonstrated in immunoelectrophoretic pattern with infected rat sera, that at least 2 examinations by the IHA test should be done at certain intervals of time to determine whether antibody levels increase. In other words a rise in IHA antibody titers is the most reliable serologic diagnosis.

To obtain more epidemiological information of the disease in the Ryukyu Islands, a skin test survey was conducted among inhabitants of Okinawa, Miyako and Ishigaki and the results presented in Table 11. A total of 335 persons were tested and positive reactions were obtained in 130 or 36.4%. The positive rates in Miyako (66.7% in the Karimata and 45.7% in the Nishihara area) where half (6 cases) of total clinical cases in the Ryukyu Islands were reported, were higher than those of the other islands (23.6% in Ishigaki and 22.6% in Naha). Similar results were obtained in a survey conducted in Taiwan, where positive rates among inhabitants of the southern and eastern areas were higher than those of the northern and central areas. Another survey using the IHA test was conducted in the Ryukyu Islands (Otsuru *et al.*, 1977b) and sera from 2,937 persons collected randomly in 20 areas were tested. Included among them were the sera of 135 persons who were skin-tested in the Nishihara area of Miyako. As seen in Table 12, 9.3% to 63.2% of the sera had antibody titers of 1:16 or greater in every area, but only 1.7% of the control group (Niigata Prefecture) had titers ≥ 1:16. The positive rates in Okinawa were generally higher among inhabitants in agricultural districts of the northern areas than those of the central and southern areas.

Table 11

Skin test in inhabitants of the Ryukyu Islands comparing results with other areas

Area		No. examined	No. classified by size of wheal ≦7mm (−)	7-9mm (±)	≧9mm (+)	Positive rate (%)
Naha (Okinawa)		106	66	16	24	22.6
Nishihara (Miyako)		138	53	22	63	45.7
Karimata (Miyako)		39	7	6	26	66.7
Ishigaki (Ishigaki)		72	37	18	17	23.6
		136*	115	8	13	9.6
Niigata (Honshu)		108	89	22	7	9.5
Taiwan	Northern	306	260**		46	15.0
	Central	161	138**		29	18.0
	Southern	626	395**		231	36.0
	Eastern	384	192**		192	50.0

* Students of Niigata University School of Medicine

** No. of cases which showed the wheal<9mm

In the Kushi and Nakijin areas, the positive rates were as high as 53.1% and 63.2%, respectively. High positive rates were also obtained in the Nishihara (45.2%), Irabu (53.1%) and Hirara (48.0%) areas of Miyako, and among each of them the ratio of titers over 1:128 were 57.4% of Nishihara and 44.1% of Irabu. In other islands, however, the positive rates were not as high showing 25.2% in Ishigaki, 26.1% in Kume and 14.6% in Iejima. Sera collected from 61 persons who had IHA positive antibody levels in the Nishihara area of Miyako were tested for cross-reactivity against the other parasite antigens by the Ouchterlony method. Clear precipitin bands were observed between some sera and the antigens of *Dirofilaria immitis* and/or *Ascaris lumbricoides*. The IHA tests were repeated with these sera absorbed with immunoadsorbent gels coupled with the antigens of *D. immitis* and *A. lumbricoides*. The number of positive sera with titers over 1:16 against *A. cantonensis* decreased to 33 (24.4%) after the absorption, and occurred mainly in the group of persons having histories of habitual or occasional contacts with the intermediate or paratenic hosts. In a similar survey on Taiwan with the same antigens, positive IHA rates in endemic areas such as the southern and eastern parts of the island were higher than those in the northern and central areas. The results suggested that most of the inhabitants showing positive immunologic reactions might have been infected unknowingly with the parasite or the diagnoses were incorrect. This was also suggested for results obtained in an examination of sera from suspected cases of Japanese encephalitis (JE) on Taiwan (Otsuru *et al.*, 1977b). During the period from 1972 to 1975, sera from 690 clinically suspected cases of JE were examined serologically for hemagglutination inhibition (HI) antibody for JE virus. A total of 658 sera were also examined by IHA for angiostrongyliasis. The results are summarized in Table 13. The IHA positive rate of the HI negative (excluded) group was significantly higher (46.9%) than those of equivocal (7.8%) and confirmed (3.4%) groups. Furthermore, when the IHA positive rates were arranged yearly, the highest rate was always obtained in the negative group. An apparent geographic difference was also recognized in distribution of the IHA positive persons. The high positive rates were seen in the southern (23.8%) and eastern (15.3%) areas where angiostrongyliasis is endemic, while in the northern and central areas the rates were as low as 8.6% and 8.1%, respectively. These results suggest that a considerable proportion of cases of meningoencephalitis of unknown etiology may be caused by *A. cantonensis* infection or mistaken for the other diseases such as Japanese encephalitis.

Table 12

IHA test on inhabitants of the Ryukyu Islands compared with those from other areas

Area		No. examined	No. classified by reciprocal IHA titer						Positive rate(%)*
			<16	16	32	64	128	≥256	
Kunigami (Okinawa)		94	71	6	4	6	3	4	24.5
Higashi (Okinawa)		148	108	14	9	5	4	8	27.0
Nakijin (Okinawa)		68	25	9	5	21	5	3	63.2
Kushi (Okinawa)		162	76	8	17	25	10	26	53.1
Ginoza (Okinawa)		140	88	8	12	13	8	11	37.1
Onna (Okinawa)		90	78	3	1	0	1	7	13.3
Yomitan (Okinawa)		279	216	13	11	18	10	11	22.6
Nakagusuku (Okinawa)		120	89	6	8	6	3	8	25.8
Sajiki (Okinawa)		44	29	4	2	3	2	4	34.1
Naha (Okinawa)		393	348	6	1	14	20	4	11.5
Haebara (Okinawa)		95	79	4	2	4	3	3	16.8
Tamagusuku (Okinawa)		75	68	1	2	0	1	3	9.3
Kanegusuku (Okinawa)		82	61	1	3	5	6	6	25.6
Nago (Okinawa)		194	173	6	3	2	5	5	10.8
Ie (Iejima)		96	82	4	2	4	4	0	14.6
Kume (Kumejima)		272	201	21	17	15	5	13	26.1
Nishihara (Miyako)		135	74	8	3	15	6	29	45.2
Irabu (Miyako)		64	30	8	8	3	2	13	53.1
Hirara (Miyako)		100	52	31	3	5	2	7	48.0
Ishigaki (Ishigaki)		286	214	21	12	18	7	14	25.2
Niigata (Honshu)		242	238	3	1	0	0	0	1.7
Taiwan	Northern	179	143	13	5	10	3	5	20.1
	Centarl	218	137	11	9	7	3	7	17.0
	Southern	221	159	21	12	9	7	13	28.1
	Eastern	283	181	35	18	11	9	29	36.0

* The sera with antibody titer over 1:16 were regarded as positive.
Specimens from Taiwan were a male group of 20-years-old examined for military service.

Table 13

Incidence of positive reaction of IHA test for angiostrongyliasis among suspected Japanese encephalitis (JE) cases, in relation to the results of serological examinations for JE by years and areas on Taiwan

	JE group	Confirmed		Equivocal		Excluded		Total	
		No. of cases examined	No. of positive (%)	No. of cases examined	No. of positive (%)	No. of cases examined	No. of positive (%)	No. of cases examined	No. of positive (%)
Year	1972	98	5 (5.1)	81	6 (7.4)	41	20 (48.7)	220	31 (14.1)
	1973	50	2 (4.0)	65	8 (12.3)	32	13 (40.6)	147	23 (15.6)
	1974	102	2 (1.9)	94	4 (4.2)	18	6 (33.3)	214	12 (5.6)
	1975	42	1 (2.3)	28	3 (10.7)	7	7 (100)	77	11 (14.3)
Area	Northern	173	4 (2.3)	155	7 (4.5)	54	22 (40.7)	382	33 (8.6)
	Central	43	1 (2.3)	50	3 (6.0)	18	5 (27.7)	111	9 (8.1)
	Southern	49	3 (6.1)	41	7 (17.0)	23	17 (73.9)	113	27 (23.8)
	Eastern	27	2 (7.4)	22	4 (18.1)	3	2 (66.6)	52	8 (15.3)
	TOTAL	292	10 (3.4)	268	21 (7.8)	98	46 (46.9)	658	77 (11.7)

On Taiwan, 12 cases of angiostrongyliasis or eosinophilic meningoencephalitis (Cross, 1967) and 125 cases of eosinophilic meningitis or meningoencephalitis caused probable by *A. cantonensis* (Yii *et al.*, 1975; Yii, 1976) were reported. Many of these patients were aborigines especially children and nearly all had contact with a molluscan intermediate host. In order to evaluate the usefulness of the skin test for diagnosis of the disease, Suzuki *et al.* (1974) carried out tests on 21 cases of eosinophilic meningoencephalitis which occurred in the southern and eastern Taiwan using the same antigen employed in our skin testing studies. Six of 7 cases in which the time between the first medical examination and the skin test was within 10 days, showed reactions below 9 mm in the mean wheal size, whereas 11 out of 14 cases (71.4%) in which the time between examination and test was more than 1 month the reactions were 9 mm or greater. From these results he suggested that the antibody responsible for the skin test could be detected 10 to 30 days after the onset of the disease. In Thailand, Kamiya (1972) also examined 15 suspected cases of angiostrongyliasis by hemagglutination and immunoelectrophoresis and most showed positive responses.

In addition to these immunological examinations on human cases, immunological aspects in *A. cantonensis* infection in experimental animals have been investigated in Japan. The antibody productions in these animals were demonstrated by a variety of immunological methods, such as immunodiffusion (Kamiya and Kanda, 1973; 1975; Sekikawa *et al.*, 1973; Yoshimura *et al.*, 1977), hemagglutination test (Kamiya and Tanaka, 1969; Kamiya and Kanda, 1973, 1974; Sato, 1975; Yoshimura *et al.*, 1977) and indirect fluorescent antibody test (Ishii and Kamiya, 1972). Reaginic antibody productions in rabbits and rats infected with *A. cantonensis* were also demonstrated by Yoshimura and Yamagishi (1976). Sato *et al.* (1978) detected antigenic components of *A. cantonensis* in immune complexes in the sera of infected rats. The positive micro-precipitation reactions on living larvae and eggs were reported as a method for the diagnosis by Oku *et al.* (1978). Yoshimura and his co-workers studied cellular responses in infected animals (Yoshimura and Soulsby, 1976; Yoshimura and Yamagishi, 1975; Yoshimura *et al.*, 1976a, b) and developed a method to surgically transplant young adult worms from rat brains to the pulmonary arteries of normal animals (Yoshimura *et al.*, 1978). This method seems to be useful for analysis of stage specific immune responses as well as of development in abnormal hosts. Watanabe *et al.* (1978) attempted to culture the cells from sex glands of adult females and successfully used the cultured cells as an antigen for the indirect fluorescent antibody test.

Recently, physiological and biochemical investigations on the metabolism of this parasite have been carried out by Japanese researchers (Yanagisawa *et al.*, 1967-1971; Maki and Yanagisawa, 1975-1978; Oguchi *et al.*, 1978). The results of these studies may be applied to the diagnosis and treatment of the disease in the near future.

MODE OF INFECTION

Alicata and Jindrak (1970) proposed that the distribution of *A. cantonensis* was in accordance with the introduction of the giant African snail, *A. fulica*. The snail shell often grows over 10 cm in the height and because of the migratory habit, rapacity, and high susceptibility to the parasite, it may harbor tens of thousands of the infective larvae of

A. cantonensis. The snail seems to have gradually spread from eastern Africa to the Pacific regions. This gradual extension of distribution and the occurrence of rodent or human infections with the parasite appears to be correlated geographically and periodically. On Taiwan, the snail was introduced as a food in 1932, the adult *A. cantonensis* was detected in rodents in 1937 and the first human case occurred in 1944.

In the Ryukyu and Amami Islands, the snail was introduced, possibly, from Taiwan as a medicine for asthma between 1935 and 1940 and from the South Pacific Islands as a food during World War II. Rodent and human infections with the parasite were reported in 1964 and in 1969, respectively. Although the snail was consumed as food during and after World War II in the Ryukyu Islands, this habit is not practiced at the present time and, because of its damage to vegetables, control measures have been implemented on many islands. On Miyako the snail is used as food for eel and prawn cultivation. In addition, the inhabitants have a custom of using live slugs and fresh liver of frogs or toads in the treatment for asthma. These practices of course have epidemiological significance in human *Angiostrongylus* infections.

Whether third-stage larvae can be released from the intermediate or paratenic hosts naturally and whether the larvae can invade the skin is important in transmission of the parasite to man. According to recent observations in Okinawa (Asato and Kishimoto, 1976b), third-stage larvae of *A. cantonensis* were not released from *A. fulica* living or dead, as long as the body was not damaged. In the case of *L. alte,* the larvae began to escape from the snail within several hours after death with more than half of the larvae being shed 24 hours after death. If the mantle or intestine of *A. fulica* was damaged, active larvae were released during a period for about a week in water at 10°-20° C. The released larvae were capable of infecting rats orally or through lacerated or intact skin, similar to findings by Intermill *et al.* (1972). The rate of infection through the skin, however, was relatively low. According to Cheng and Alicata (1964), infective larvae of *A. cantonensis* were observed to escape in small numbers from snails and slugs immersed in water, especially injured molluscs. These results suggest that infection can possibly be acquired by eating raw vegetables bearing the small intermediate or paratenic hosts or the larvae shed or passed from snails, slugs or paratenic hosts, and that the infection through skin may also occur under suitable condition.

SUMMARY

The medico-zoological findings on the definitive, intermediate and paratenic hosts for *Angiostrongylus cantonensis* and human infection in Japan have been described on the basis of research reports by Japanese workers and from data obtained from the surveys conducted by the Medical Zoology Department, Niigata University. The findings were related more to the Ryukyu Islands, the Southwest (Nansei) Islands of Japan, in addition to neighboring Taiwan.

In the Ryukyu and Amami Islands, the giant African snail, *Achatina fulica,* was introduced from Taiwan in 1935 to 1940 and from the South Pacific Islands during World

War II. The adult and larva of *A. cantonensis* have frequently been found in wild rats and mollusks including *A. fulica* since 1964 mainly in the Ryukyu Island. Twelve human cases of eosinophilic meningoencephalitis probably due to *A. cantonensis* have been reported since 1969, 30 years after introduction of the snail.

Immunological diagnosis was carried out on some patients, while the immunological surveys were conducted among the inhabitants. The source of infection of angiostrongyliasis has been investigated by several workers, and *A. cantonensis* has been found in a large number of rats and several species of snails and slugs of the Ryukyu Islands. The infection rates are as high as those reported in Southeast Asia and the Pacific Islands. Infective-stage larvae were also found in frogs and toads and the medicinal use of *Bufo asiaticus* has been associated with 2 human cases.

The distribution of the parasite has been extended along the northern parts of the Southwest Islands further to the southernmost areas of Kyushu. Since 1968 the parasite has been recovered from rats and slugs in the middle of Honshu and from rats in Hokkaido. These findings suggest the possibility of human angiostrongyliasis occurring in many parts of Japan.

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EXPERIMENTAL STUDIES ON *ANGIOSTRONGYLUS* SPECIES AND STRAINS IN MONKEYS AND LABORATORY ANIMALS*

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Until a few years ago Taiwan was the only endemic area for *Angiostrongylus cantonensis* reporting the recovery of larvae of this parasite from the cerebrospinal fluid (CSF) of patients with eosinophilic meningitis or eosinophilic meningoencephalitis. Similar findings have since been made from Thailand and Malaysia, but only on rare occasions. Over 250 suspected and confirmed cases of angiostrongyliasis have been reported on Taiwan since the first report by Nomura and Lin (1945), and larval forms of the parasite have been recovered from nearly 10% of the cases (Chen, 1978; Cross, 1978). In experimental studies conducted at NAMRU-2 with the Taiwan strain of *A. cantonensis* it was shown that the oral administration of large numbers of *A. cantonensis* larvae to the Taiwan monkey *(Macaca cyclopis)* resulted in death of the animal (Wood, 1965, unpublished), however in similar studies in Malaysia using a Malaysian strain of *A. cantonensis* and Malaysian monkeys, the animals survived heavy infections (Heyneman, August 1966, personal communication). The presence of *A. cantonensis* larvae in CSF of patients in Taiwan, the finding that Taiwan monkeys died with large numbers of *A. cantonensis* while monkeys in Malaysia survived, prompted studies on the pathogenicity of the Taiwan strain of *A. cantonensis* in Taiwan monkeys and studies to determine whether differences existed between geographic strains of the nematode. The results of these studies will be reported here along with studies undertaken on host parasite relationships and acquired immunity in monkeys and rats. Brief mention will also be made of experimental studies of strains and species of *Angiostrongylus* in other monkey species and laboratory animals.

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The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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MATERIALS AND METHODS

Eight strains of *A. cantonensis* used were kindly provided by investigators from several geographic area. Table 1 lists the strains, the intermediate molluscan hosts from which the strains originated, and the persons providing the strains. The larvae were isolated from the molluscs by standard methods of homogenizing the molluscan tissue, digestion in artificial gastric juice and recovery from a Baermann apparatus. The nematode strains were established in Long-Evans (hooded) rats and maintained in the laboratory by passage through *Biomphalaria glabrata* and rats. Infections were introduced with water into animals by stomach tube in dosages of 50 larvae for rats and approximately 10,000 for monkeys. Rats and other laboratory animals were from colonies maintained at NAMRU-2 and the monkeys were originally obtained from animal dealers on Taiwan and retained in the laboratory for 6 to 12 months or longer prior to use. Both male and female adult monkeys between 4-5 kg body weight were used.

Table 1

Sources of geographic strains of *Angiostrongylus cantonensis*

Strain	Host	Provided by
Taiwan	*Achatina fulica*	NAMRU-2
Thailand	*Pila sp.*	Dr. Chamlong Harinasuta Mahidol University, Bangkok
Hawaii	*Biomphalaria glabrata*	Dr. Leon Rosen Pacific Research Section National Institutes of Health Honolulu
Indonesia	*Achatina fulica*	Dr. Sri Margono University of Indonesia, Jakarta
Viet Nam	*Achatina fulica*	Dr. Sheldon White U. S. N. Preventive Medicine Unit Danang
Philippines	*Vaginulus plebeius* *Achatina fulica*	Dr. Niela Salazar Institute of Public Health University of the Philippines, Manila
Malaysia	*Bradybaena similaris* *Microparmarion malayanus* *Laevicaulus alte*	Dr. Boo-liat Lim Institute for Medical Research Kuala Lumpur
Okinawa	*Achatina fulica*	Dr. Tom Simpson Okinawa Central Hospital Okinawa, Japan

One to 2 days prior to infection and biweekly thereafter blood and CSF were obtained from the monkeys and examined by standard methods for changes associated with the infection. Body temperatures were followed and white blood counts done on blood and CSF. Protein, glucose and chloride determinations were also done on CSF. Monkeys that died were necropsied and examined for the presence of the parasite and for pathologic changes. Tissues from the major organs were studied histologically. Rat controls were killed when the monkeys died and examined for the parasite.

In studies on host parasite relationships and acquired immunity rats and other laboratory animals were infected with known numbers of larvae and killed at various times. Methods varied with experiments and details will be presented with the results.

RESULTS

Pathogenesis of the Taiwan strain of *Angiostrongylus cantonensis* in monkeys. The Taiwan strain of *A. cantonensis* was found to be highly pathogenic to Taiwan monkeys. Nine animal were given 10,000 larvae of *A. cantonensis* and all died as a result of the infection between 7 and 35 days (Table 2). Prior to death the animals refused food and lost from 0.5 to 2 kg body weight depending on the length of survival. Some animals became listless and collapsed in their cages and some developed flaccid-hindquarter paralysis. Most developed elevated body temperature of 2-3° F and lower temperatures when nearing death. Peripheral blood leukocytosis developed in most animals and ranged from 11,000-17,000/cu mm but without marked elevations in peripheral eosinophiles. Leukocytosis was greatest in most animals 2 weeks postinfection. Pre-infection leukocyte levels ranged from 7,000-11,000/cu mm.

Table 2

Pathogenesis of the Taiwan strain of *Angiostrongylus cantonensis* in the Taiwan monkey *Macaca cyclopis*

Signs, symptoms and necropsy findings after exposure to 10,000 larvae.

1. **Loss of appitite and body weight.**
2. **Leukocytosis without marked eosinophilia.**
3. **Spinal fluid cloudy, eosinophilic pleocytosis.**
4. **Worms on occasion in spinal fluid.**
5. **Hindquarter flaccid paralysis.**
6. **Death 7-35 days.**
7. **Vascular congestion of all organs.**
8. **Worms in leptomeninges, brain and cord.**

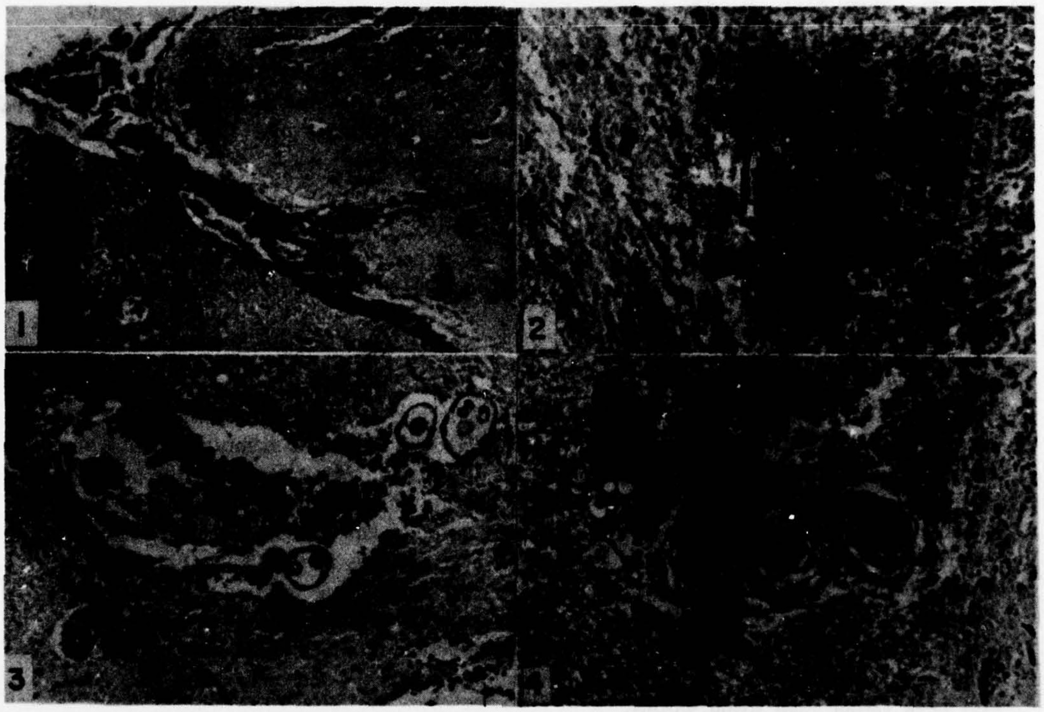
The CSF from all animals was cloudy and contained erythrocytes, neutrophiles, lymphocytes and eosinophiles. Eosinophilic pleocytoses were usually highest, 200-700/cu mm between 14 and 28 days postinfection. On a few occasions third-and-fourth-stage *A. cantonensis* larvae were recovered from the spinal fluid. CSF protein, glucose and chloride levels varied during the course of the disease from 19-96 mg%, 30-180 mg% and 120-135 mEq./L, respectively.

Generally at autopsy the lungs were found to be atelectatic and there was vascular congestion of all organs, including the central nervous system (CNS). *A. cantonensis* larval and young adult forms were found in the leptomeninges, brain and spinal cord but the parasite was not found in the lungs or other organs. Death was attributed to meningitis and or myelitis.

Necropsies were done on all animals but for brevity only CNS histological findings from representive animals will be presented. In a monkey that died 7 days after infection there was vasculature congestion in the leptomeninges (Fig. 1) but very little tissue reaction was seen. Similarly in an animal that died after 13 days there was hemorrhage into the subarachnoid space in the cerebral hemisphere but little reaction associated with the parasite. Figure 2 is a section of spinal cord showing the parasite in the central canal with very little reaction. In a monkey that died at 24 days the cerebral leptomeninges were congested; lymphocytes and a few neutrophiles were found near parasites in the tissue (Fig. 3). In a second monkey that died after 24 days a great deal more tissue reaction was seen with eosinophiles, plasma cells, lymphocytes and foreign body giant cells. Similar results were observed in an animal that died at 32 days postinfection in which there was perivascular hemorrhage, lymphocytes and giant cell formation (Fig. 4). Giant cells were associated with dead rather than living worms.

Worms were recovered from the brain and cord of most animals necropsied. In early infections most parasites were in the third larval stage and ranged in size from 0.34-4.4 mm. After one week a few fourth-stage larvae were found and were 0.64-0.72 mm in length. Worms after two weeks in the monkey were fourth-stage and ranged in size from 1.2-2.2 mm. At three weeks fourth-stage larvae and young adults were found and measured 3.1-6.1 mm. After four weeks the worms were still fourth-or-fifth-stage and measured 4.8-9.8 mm. Worms in rats at this time were in the lungs and in the adult stage, varying in length from 11.7-16.3 mm. Worms were never found in the lungs of monkeys.

Since it had been established that 10,000 larvae of *A. cantonensis* would cause death in Taiwan monkeys, additional experiments were done to determine the pathogenesis at lower larval dosages. One monkey each was given approximately 500, 800, 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000 and 10,000 larvae of the Taiwan strain. All monkeys developed an eosinophilic pleocytosis and monkeys given 5,000, 7,000, 9,000 and 10,000 larvae died at 32, 21, 14 and 32 days postinfection, respectively. Worms were recovered from the CNS of all animals and the histopathologic findings were similar to those reported above at the respective days. These studies showed that while some animals died when given lower numbers of larvae, larger dosage were generally required to kill the animal. All of the monkeys surviving these infections were later given 10,000 larvae of the Taiwan strain to determine if immunity was acquired. Although all developed eosinophilic pleocytoses, all survived the challenging infection; control monkeys given 10,000 larvae died.



Figures 1-4. Histological sections of the central nervous system of monkeys with experimental angiostrongyliasis (H & E, X100). (1) Brain of monkey 7 days postinfection showing vascular congestion, but little tissue reaction. (2) Cord of monkey 13 days post-infection showing a cross-section of a worm in the central canal but little tissue reaction. (3) Cross-section of *A. cantonensis* in the cord substance of a monkey that died 24 days after infection; cellular infiltration was evident. (4) Foreign-body giant cell formation around *A. cantonensis* larva in the cord substance of a monkey that died 32 days after infection with 10,000 third-stage larvae.

Rats were also given varying numbers of larvae to determine lethal dosages. Six rats were each given 100, 150, 200, 250, 300, 350, 400, 450, 500, 800 and 1,000 larvae. As expected the survival time decreased as the larval doses increased. Rats receiving 100 and 150 larvae survived significantly longer than rats receiving 200 larvae and above; i.e. rats given 100 larvae survived an average of 204 days, 150 larvae 131 days, 200 larvae 65 days and above 500 larvae less than 29 days.

Pathogenesis of geographic strains *Angiostrongylus cantonensis* in the Taiwan monkeys. While the preceeding studies were underway strains of *A. cantonensis* were obtained from Thailand, Hawaii, Indonesia, Viet Nam, Philippines, Okinawa and Malaysia. Once established in the laboratory, approximately 10,000 larvae from each strain were introduced into Taiwan monkeys and the animals followed. Additional monkeys were also infected with the Taiwan strain for comparison. Table 3 lists the number of monkeys exposed to infection, the number that died and the days of survival after infection. All animals given the Taiwan, Thailand, Hawaiian, Indonesian, Viet Nam, Philippine and Okinawan strains of the parasite died. Some animals survived the infection for only a week, while others lived for several weeks. The animals manifested symptoms similar to those reported above for monkeys given the Taiwan strain. The blood, CSF, gross and histo-pathological findings were also similar. In other words, the pathogenesis of strains of *A. cantonensis* from Thailand, Hawaii, Indonesia, Viet Nam, the Philippines and Okinawa was similar to the Taiwan strain in Taiwan monkeys.

Conversely, the results obtained from monkeys exposed to the Malaysian strain of *A. cantonensis* were different. Seven Taiwan monkeys were given 10,000 larvae of this strain but none died. The animals lost weight and developed an eosinophilic pleocytoses but all recovered completely after 3 or 4 weeks.

Table 3

Survival time of Taiwan monkeys given 10,000 larvae of geographic strains of *Angiostrongylus cantonensis*

Geographic strain	Number monkeys infected	Number died	Number survived	Days of survival
Taiwan	18	18	0	6-35
Thailand	3	3	0	11-12
Hawaii	5	5	0	6-16
Indonesia	2	2	0	7-11
Viet Nam	2	2	0	6-28
Philippines	2	2	0	6-12
Okinawa	4	4	0	6-20
Malaysia	7	0	7	-

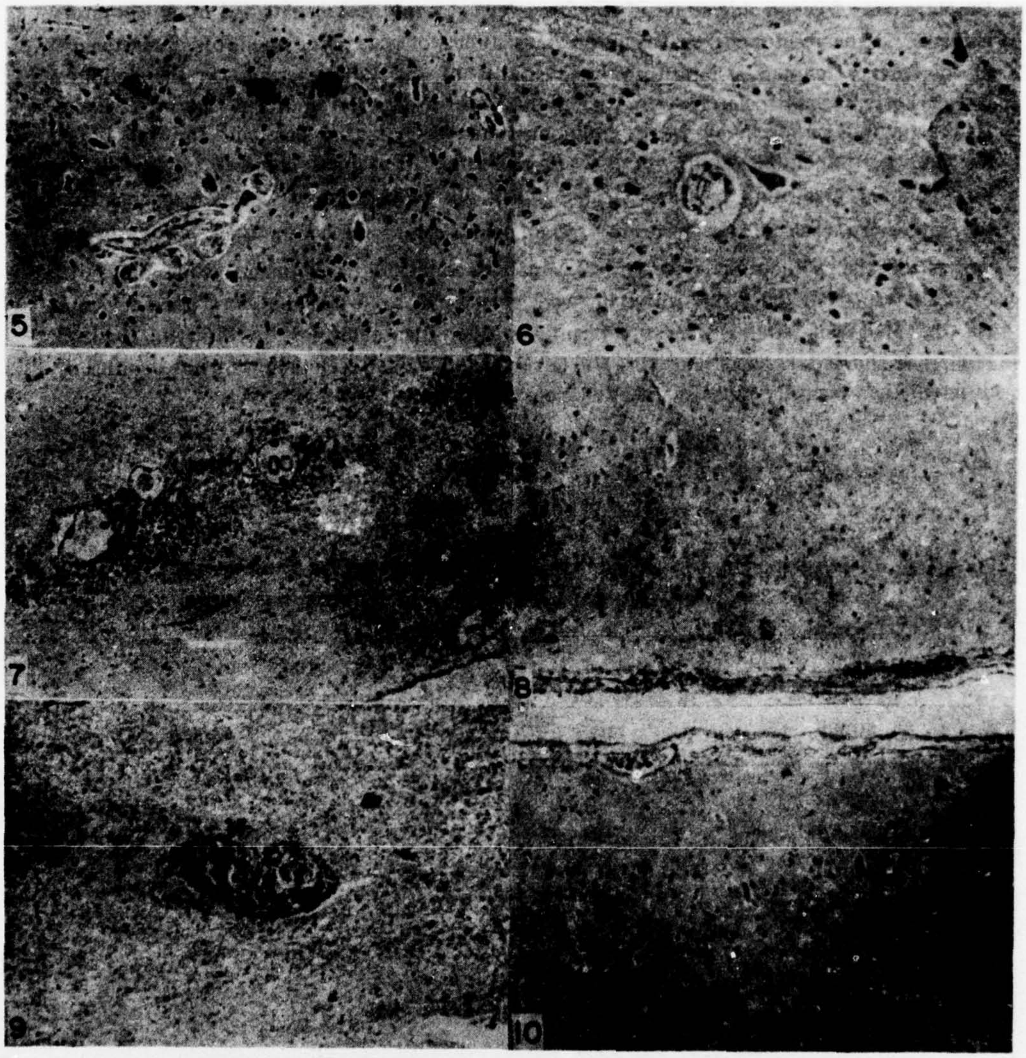
Further studies with the Malaysian strain of *Angiostrongylus cantonensis* in the Taiwan monkey. As a result of the above findings additional studies were done on the pathogenesis of *A. cantonensis* from Malaysia in the Taiwan monkey. First a monkey was infected with 10,000 larvae of the Malaysian strain and killed 14 days after infection to determine whether the worms migrated to the CNS. At necropsy the CNS was congested and lymphocytes were present. Third-and-fourth-stage larvae, 0.75-4.1 mm in length, were recovered from the brain and cord. Worms from the brain of control rats were fourth-stage and ranged in size from 2.0-7.8 mm.

In another experiment, two monkeys were each given 20,000 larvae and the animals followed. There were no symptoms and little change in peripheral leukocytosis and body temperature, but eosinophilic pleocytoses occurred. Protein and glucose levels in the CSF became elevated but decreased after 2-4 weeks and chloride levels changed very little. The eosinophiles disappeared from the CSF after one month and both animals survived.

Subsequently, a comparative study was done whereby 3 monkeys each were given 10,000 larvae of either the Taiwan or Malaysian strain of *A. cantonensis* and one monkey from each group killed at 10, 20 and 30 days postinfection. Eosinophilic pleocytoses developed in all animals and there were increases in CSF protein and glucose. At 10 days postinfection over 500 third-and-fourth-stage larvae of the Taiwan strain were recovered from the brain and cord of the monkey at necropsy. The larvae ranged in size from 1.3-2.3 mm. A mild meningeal reaction with a few eosinophiles was seen in the tissue (Fig. 5). In the monkey given the Malaysian strain only 7 third-stage larvae were recovered from the CNS and these measured only 0.4-1.5 mm in length. Very little tissue reaction related to the infection was seen in the CNS (Fig. 6). At 20 days postinfection 34 fourth-stage larvae (2.9-7.3 mm in length) were recovered from the CNS of the monkey given the Taiwan strain and a severe meningeal reaction was seen along with an out-pouring of eosinophiles and lymphocytes (Fig. 7). Although no parasites were recovered from the monkey given the Malaysian strain and killed at the same time, a mild meningeal reaction was seen with a slight infiltration of lymphocytes and eosinophiles (Fig. 8). The monkey given the Taiwan strain and killed at 30 days had a severe meningeal reaction with giant cell granuloma formation containing eosinophiles (Fig. 9). Only 5 living worms were recovered from the brain and these measured 4.8-9.8 mm in length. No worms were found in the CNS of the monkey given the Malaysian strain and no evidence of meningitis was observed (Fig. 10).

Acquired immunity in monkeys given the Malaysian strain. A series of experiments were also done to determine whether monkeys once infected with the Malaysian strain of *A. cantonensis* were susceptible to a challenging infection with one of the pathogenic strains. The seven monkeys initially infected with the Malaysian strain along with control monkeys, not previously exposed to infection, were given 10,000 larvae of either the Taiwan, Thailand or Hawaiian strains. Although all monkeys previously exposed to the Malaysian strain developed eosinophilic pleocytoses, none died as a result of the challenging infection. The control monkeys, however, died within 2 to 3 weeks. Some of the monkeys initially given the Malaysian strain were later challenged with equally large larval dosages of a pathogenic strain, a second and third time, and all animals survived the challenges. After the first challenge there was little eosinophilic pleocytoses suggesting that the worms on second and third challenge failed to reach the CNS.

Conclusions on the Malaysian strain of *Angiostrongylus cantonensis*. The results of these studies, at this point in the investigation, indicated that the Malaysian strain was indeed different from the other geographic strains of *A. cantonensis* tested. Although the parasite was capable of migrating to the CNS in monkeys in small numbers and was able to cause some disease, death did not result from the infection. Furthermore, the strain was able to immunize monkeys against the lethal effects of high numbers of larvae of geographic strains of the parasite shown to be pathogenic to monkeys.



Figures 5-10. Histologic sections (H & E, X100) of central nervous system tissue from monkeys given 10,000 larvae of *A. cantonensis* or *A. malaysiensis* and killed at 10, 20 and 30 days. (5) Section of the brain of a monkey given *A. cantonensis* and killed 10 days postinfection; a mild reaction with some eosinophiles were present at this time. (6) Little reaction is associated with *A. malaysiensis* larva in the spinal cord at 10 days. (7) A severe meningeal reaction with eosinophiles is evident around *A. cantonensis* larvae in brain tissue of a monkey killed at 20 days. (8) Only a mild meningeal reaction is seen in brain tissue, from a monkey given *A. malaysiensis* and killed at the same time. (9) Spinal cord from a monkey given *A. cantonensis* and killed at 30 days showing a giant cell granuloma. (10) Brain of monkey given *A. malaysiensis* and killed at 30 days; no evidence of meningitis was seen.

Development of geographic strain of *Angiostrongylus cantonensis* in rats and other laboratory animals. The results obtained from previous studies of geographic strain of *A. cantonensis* in monkeys prompted a return to basic studies of the various strains in the definitive rodent host. Twenty male rats were each given 50 third-stage larvae of the Taiwan, Thailand, Hawaiian, Indonesian, Viet Nam, Philippine and Malaysian strains and the rats killed 42 days postinfection. Adult worms were recovered from the pulmonary vessels of the rats, counted, fixed and measured. Table 4 lists the percentage of worms recovered from the rats given each of the respective geographic strains and shows that 70-82% of the worms of the pathogenic strains were able to complete development while only 61% of the larvae from the Malaysian strain were able to do so. While the worm recovery rates for the Malaysian strains were significantly smaller than rates for the Taiwan, Thailand, Philippine and Indonesian strains ($P<.01$) (Kruskal and Wallis Test), they were not significantly different than recovery rates for the Viet Nam and Hawaiian strain ($P<.05$).

Table 4

Development of geographic strains *Angiostrongylus cantonensis* in the laboratory rat given 50 larvae: adult worm recoveries

Geographic strain	No. of rats	Percentage* of worms recovered: Range	Mean
Taiwan	20	38-92	82
Thailand	20	58-92	75
Hawaii	20	42-84	73
Indonesia	20	62-94	80
Viet Nam	20	46-82	70
Philippines	20	66-94	80
Malaysia	20	26-92	61

* To nearest whole number.

Total lengths of 70-80 male and female worms from each strain recovered from rats were determined and both males and females of the Malaysian strain were found to be significantly smaller ($P< .001$) than worms from the other geographic strains (Table 5). The spicules of the males from each strain were also measured (Table 6) and those from males of the Malaysian strains were significantly smaller ($P< .001$). Conversely, the spicules of the males of the Thailand strain were significantly larger ($P< .001$). (Spicular size was shown by Bhaibulaya, 1968, to be an important taxonomic character in describing *A. mackerrasae* as a new species).

Table 5

Geographic strains of *Angiostrongylus cantonensis* in laboratory rats: measurements of adult worms*

Geographic strain	Length of females (mm) Range	Mean	Length of males (mm) Range	Mean
Taiwan	15.8-24.0	21.1	13.1-20.0	17.7
Thailand	17.7-24.8	22.4	13.4-19.3	17.6
Hawaii	18.3-25.5	21.9	14.5-18.6	16.3
Indonesia	17.3-23.5	20.9	14.2-17.8	16.2
Viet Nam	19.0-25.2	22.2	14.2-19.9	16.8
Philippines	16.5-23.9	21.3	14.5-17.5	15.9
Malaysia	15.6-23.4	19.2	13.4-16.6	15.3

*70 to 80 of each sex measured.

Table 6

Geographic strains of *Angiostrongylus cantonensis* in laboratory rats: length of spicules (mm)

Geographic strain	No. measured	Range	Mean
Taiwan	328	1.10-1.47	1.25
Thailand	307	0.96-1.46	1.32
Hawaii	319	0.97-1.45	1.22
Indonesia	319	1.06-1.39	1.26
Viet Nam	266	1.07-1.43	1.26
Philippines	384	0.94-1.35	1.18
Malaysia	272	0.88-1.44	1.01

Other laboratory animals such as mice, hamsters, Mongolian gerbils, guinea pigs and rabbits were given 50 larvae of the Malaysian strain, but the parasite was not able to complete development in these hosts. Most animals died within 1-2 weeks and larval worms were found in the brains. A few of these animals survived but no worms were found in the CNS, lungs or elsewhere at necropsy. Adult worms were recovered from the lungs of rats serving as controls for each infection and killed 42 days postinfection. In a companion study with the Taiwan strain of *A. cantonensis* similar results were obtained on the development of the parasite in the above animals. All animals died with larval forms found in the brains but in a few hamsters young-adult worms were recovered from the lungs at 25 days post-infection. Additional studies were done with hamsters whereby 10 animals each were given 25 larvae of each of the geographic strains. All animals died between 16-30 days and while most worms were found in the brains, 1-5 worms were recovered from the lungs of a few hamsters receiving the Malaysian, Taiwan and Philippine strains.

Confirmation of a new species; ***Angiostrongylus malaysiensis.*** While the studies on geographic strains of *A. cantonensis* were becoming finalized discussions were held with Dr. Manoon Bhaibulaya, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand on the possibility of the Malaysian strain being a new species of *Angiostrongylus.* After careful study of the morphologic features by Dr. Bhaibulaya, the Malaysian strain was described as a new species, *A. malaysiensis* (Bhaibulaya and Cross, 1971). To validate the status of the species we undertook cross-breeding experiments whereby single third-stage larvae of *A. cantonensis* and *A. malaysiensis* were fed to laboratory rats. Infection developed in one rat and the F_1 hybrid larvae, after passage in snails, were fed to rats. Patent infections did not develop in the rats and at necropsy the male worms were found to be sterile. These findings indicated that *A. malaysiensis* was a valid species (Cross and Bhaibulaya, 1974).

In another hybridizing experiment we attempted to backcross the F_1 hybrid with the parent species. Twenty rats were each given one larvae of the respective species and two rats become positive. Snails *(B. glabrata)* were exposed to the first-stage larvae and either one or two of the third-stage larvae of the F_1 hybrid from these snails were given to 80 rats along with one larva of either parent species; *A. cantonensis* or *A. malaysiensis.* Patent infections developed with first-stage larvae being recovered from 9 to the 80 rats. Adult worms were found in the pulmonary vessels of most of the remaining 71 rats, but in all cases reproduction did not occur when the males were F_1 hybrids. In animals with patent infections the males were always of either one of the parent species. The hybrid females were fertile since backcross progeny were recovered from rats infected with them and males from either parental species. The results of these backcrossing experiments offer further evidence that *A. malaysiensis* is a valid species.

Development of ***Angiostrongylus mackerrasae*** **in Taiwan monkeys.** *A. mackerrasae* was obtained from Dr. Manoon Bhaibulaya from Australia. The parasite was established in Long-Evans rats and *B. glabrata* in the laboratory and on 3 different occasions Taiwan monkeys were exposed to infections with 10,000 third-stage larvae. Although a small number of eosinophiles were found in the CSF 2-4 weeks after infections the animals did not manifest symptoms of infection and all 3 survived. One animal died of other causes 3 months after infection and at necropsy no parasites were found and pathologic changes attributed to the infection were not observed. Rat controls were killed after several months and adult *A.*

mackerrasae were recovered from the lungs.

Acquired immunity in rats to *Angiostrongylus cantonensis*. Early in these investigations experiments were carried out to determine whether rats were able to acquire immunity to *A. cantonensis*. In the first experiment Long-Evans rats were given 50 larvae as an immunizing infection followed 40 days later with a challenging infection of 50 additional larvae. Control rats for each infection were also given 50 larvae. All animals were killed 40 days after the challenging and 80 days after the immunizing infections. The brains and lungs were examined at necropsy and worms recovered. Table 7 lists the results and shows that only 41% of the total (100) number of worms given was recovered from the lungs of immunized rats while 77% and 74% were recovered from the controls for the respective infections. The differences in the percentages of worms recovered from the immunized compared to both groups of control rats were significant ($P<.001$) by Wilcoxin's Test.

Table 7

Acquired immunity in rats given an immunizing infection of 50 larvae of *Angiostrongylus cantonensis* and challenged with 50 larvae of *Angiostrongylus cantonensis;* rats killed 40 days after the challenging infection

	Group I (immunized)	Group II (control)	Group III (control)
No. rats	20	8	8
Days after 1st infection	80	80	-
Days after 2nd infection	40		40
	Worms recovered	Worms recovered	Worms recovered
	Lungs	Lungs	Lungs
No. range	35-54	31-47	32-45
No. average*	41	39	37
% recovered	41%++	77%+	74%+

* Rounded to nearest whole number. + Based on 50 worms. ++ Based on 100 worms.

Since adult worms from the immunizing infection could not be distinguished from those of the challenging infection in experimental rats it was not certain whether the differences between recovery rates were due to an immunologic response against the parasites or possible due to other factors such as crowding. A second experiment was subsequently carried out whereby the immunizing and challenging infections could be distinguished

(Table 8). Rats were immunized with 50 larvae, challenged 40 days later with 50 larvae and killed 18 days after the challenging infection (a time when the larvae are in the brain). The animals were necropsied and worms recovered from the brains and lungs. Only 8% of the worms of the challenging infection were recovered from the brains of immunized rats (Group I) while in the unimmunized control rats (Group III), 78% of the worms were recovered from the brains. These differences were significantly different ($P < .001$). Eighty percent of the worms from the immunizing infection were recovered from the lungs of rats (Group I) killed 58 days after the immunizing infection and 90% of the worms recovered from the controls (Group IV) killed at the same time. However, in rats (Group II) immunized, challenged and killed 80 and 40 days, respectively, after infection a total of only 51% of the worms were recovered from the lungs. Worm recoveries from the control rats at these times were 84% (Group V) and 80% (Group VI). These results were similar to those of the previous experiment and in most cases the majority of the worms recovered from the lungs of the immunized rats were considered to be from the primary infection. These findings indicate that the parasites are able to complete migration and development in unimmunized rats, but in the immunized animals development is suppressed and the immune response appears to act against the larvae migrating to the brain.

Table 8

Acquired immunity in rats given an immunizing infection with 50 larvae of *Angiostrongylus cantonensis* and challenged with 50 larvae of *Angiostrongylus cantonensis*; rats killed 18 and 40 days after challenging infection

	Group I (immunized)			Group II (immunized)	Group III (control)	Group IV (control)	Group V (control)	Group VI (control)
No. rats		11		7	6	5	2	6
Days after 1st infection		58		80		58	80	-
Days after 2nd infection		18		40	18	-	-	40
	Worms recovered			Worms recovered	Worms recovered	Worms reocvered	Worms recovered	Worms recovered
	Brains	Lungs	Total	Lungs	Brains	Lungs	Lungs	Lungs
No. range	0-15	31-49	30-57	39-54	36-43	42-48	40-43	31-49
No. average*	4	40	44	51	39	45	42	40
% recovery	8%+	80%+	44%++	51%++	78%+	90%+	84%+	80%+

*To nearest whole number. + Based on 50 larvae. ++ Based on 100 larvae.

Acquired immunity in rats to *Angiostrongylus malaysiensis*. When it appeared that the Malaysian strain may possibly be a new species of *Angiostrongylus*, studies were done to determine whether rats could also develop an acquired immunity to this strain.

Rats were immunized with 50 larvae of *A. malaysiensis* and challenged 40 days later with 50 additional *A. malaysiensis* larvae. Controls for both infections were given the same numbers of larvae and the animals killed 20 and 42 days after the challenging infection. Table 9 presents the results. At necropsy 40% of the worms from the challenging infection were recovered from the brains while 42% of the worms from the immunizing infection were recovered from the lungs of these animals (Group I). In rats killed 82 days after the immunizing infection and 42 days after challenge (Group II) 43% of the worms were recovered from the lungs. Worm recoveries from the brains of control rats (Group III) killed 20 days after infection averaged 54% and recoveries from the lungs of controls killed 42 (Group IV) and 60 days (Group V) after infection averaged 56% and 40%, respectively. Although, the recovery rates were somewhat lower in immunized rats than in the control animals the differences in percentages of worms recovered were not significant. The findings indicate that the development of acquired immunity in rats to *A. malaysiensis*, under these experimental conditions is minimal.

Table 9

Acquired immunity in rats given an immunizing infection of 50 larvae *Angiostrongylus malaysiensis* and challenged with 50 larvae of *Angiostrongylus malaysiensis*; rats killed 20 and 42 days after the challenging infection

	Group I (immunized)			Group II (immunized)	Group III (control)	Group IV (control)	Group V (control)
No. rats		9		9	10	10	4
Days after 1st infection		60		82	-	-	60
Days after 2nd infection		20		42	20	42	-
	Worms recovered			Worms recovered	Worms recovered	Worms recovered	Worms recovered
	Brains	Lungs	Total	Lungs	Brains	Lungs	Lungs
No. range	6-36	5-32	11-60	30-53	16-31	20-38	13-25
No. average*	20	21	41	43	27	28	20
% recovery	40%$^{+}$	42%$^{+}$	41%$^{++}$	43%$^{++}$	54%$^{+}$	56%$^{+}$	40%$^{+}$

*Rounded to nearest whole number. + Based on 50 worms given. ++ Based on 100 worms given.

Cross-immunity between *Angiostrongylus cantonensis* and *Angiostrongylus malaysiensis*. An experiment was carried out to determine whether *A. cantonensis* could immunize rats against *A. malaysiensis*. Rats were immunized with 50 larvae of *A. cantonensis* and the acquired immunity challenged 40 days later with 50 larvae of *A. malaysiensis*.

The rats immunized with *A. cantonensis* were necropsied 20 days after the challenging and 60 days after the immunizing infection (Table 10). Eighteen percent of the worms from the challenging *(A. malaysiensis)* infection were recovered from the brains and 82% of the immunizing infection *(A. cantonensis)* recovered from the lung of the immunized rats (Group I). In the control rats for the challenging infection 28% of the worms *(A. malaysiensis)* were recovered from the brain at 20 days (Group II) and 20% from the lungs of rats killed at 40 days (Group III). In controls (Group IV) for the immunizing infection *(A. cantonensis)* 86% of the worms were recovered from the lungs at 60 days. Although the percentage of worms recovered in the immunized rats was lower than for the controls, the differences in recovery rates were not statistically significant. In addition, this and the preceding experiment confirms previous findings that *A. malaysiensis* does not appear to develop as readily in experimentally infected rats as *A. cantonensis*.

Table 10

Acquired immunity in rats given an immunizing infection of 50 larvae of *Angiostrongylus cantonensis* (Ac) and challenged with 50 larvae of *Angiostrongylus malaysiensis* (Am) 20 and 40 days after the challenging infection

	Group I (immunized)			Group II (control)	Group III (control)	Group IV (control)
No. rats		20		6	6	6
Days after 1st infection		60		-	-	60
Days after 2nd infection		20		20	40	-
		Worms recovered		Worms recovered	Worms recovered	Worms recovered
	Brains	Lungs	Total	Brains	Lungs	Lungs
No. range	1-19	35-49	43-67	8-17	6-17	39-48
No. average*	9	43	52	14	10	43
% recovered	$18\%^{+}$ (Am)	$82\%^{+}$ (Ac)	$52\%^{++}$ (Am Ac)	$28\%^{+}$ (Am)	$20\%^{+}$ (Am)	$80\%^{+}$ (Ac)

*Rounded to nearest whole numer. + Based on 50 Worms. ++ Based on 100 worms.

DISCUSSION

The pathogenesis of *A. cantonensis* in animals has been documented (Alicata and Jindrak, 1970) and results obtained from experimental infections in monkeys by others are similar to results reported here. In previous studies, however, the species of monkeys used were different and other workers gave much lower dosages of infective stage larvae. Loison *et al.* (1962) infected on rhesus monkey with 50 larvae per os and 50 additionsl larvae were injected intramuscularly. The monkey died at 25 days with pathologic changes found in the brain and lung. Weinstein *et al.* (1963) gave from 335-1700 *A. cantonensis* larvae to 4 rhesus monkeys and killed the animals between 17 and 69 days postinfection. The monkeys did not develop symptoms but worms were recovered from the brains. Similar to findings in the present study the parasite provoked an eosinophilic meningoencephalitis and foreign-body granulomas formed around dead worms. Worms were not found in the meninges and cord in the latter study but were present in Taiwan monkeys given much larger numbers of larvae. In another study with a rhesus monkey Alicata *et al.* (1963) gave several hundred *A. cantonensis* third-stage larvae and followed the animal for symptoms. The animal developed a respiratory illness, an increase in body temperature, leukocytosis with 7% eosinophiles and an eosinophilic pleocytosis. The animal died 3 hours after the spinal tap 25 days postinfection. The CNS was congested, and upon histologic examination cellular infiltration was observed and sections of worms seen in a granuloma. The findings are similar to those reported in this paper with infections in the same time frame.

While the present studies were being done 3 rhesus monkeys *(M. mulatta)* became available for angiostrongylid studies and each was given 10,000 third-stage larvae of *A. cantonensis;* two were given the Taiwan strain and one given the Thailand strain. All three developed a leukocytosis with eosinophilia, eosinophilic pleocytoses and elevations in CSF protein and glucose, but no change in chloride levels. The two monkeys given the Taiwan strain survived but the one given the Thailand strain developed blindness and died at 35 days postinfection. No worms were found in the CNS tissue examined at necropsy but acute and chronic inflammatory cells were seen in the meninges.

Two patas monkeys *(Erythrocebus patas)* were also exposed to approximately 10,000 larvae of *A. cantonensis;* one animal was given the Taiwan strain and the other the Philippine strain. The animal receiving the Philippine strain died at 8 days and the one given the Taiwan strain died at 21 days. Worms were recovered from the CNS of both monkeys and while little cellular response was seen in the CNS tissue of the monkey given the Philippine strain, a great deal of infiltration and chronic inflammatory cells were seen in the CNS of the monkey given the Taiwan strain.

Lim (1970) carried out experimental studies on primative primates and monkeys and gave 16 *Tupia glis* and 8 *Nycticebus coucang* 1,000 *A. cantonensis* third-stage larvae, 2 *M. fascicularis* each 1,000, 2,000 and 5,000 larvae and 4 *M. fascicularis* 10,000 larvae each. The *T. glis* usually died of the infections and *N. coucang* found to be resistant to infection. None of the monkeys died from the infection and worms were not found in the CNS of animals given 1,000-2,000 larvae and killed at 10 and 25 days, but monkeys given 5,000 larvae and killed at 10 and 15 days developed light infections. Worms were not recovered from monkeys given 10,000 larvae and killed at 5, 8 and 20 days after infection, but 2 worms were recovered from a monkey that died from other causes at 15 days. It is quite possible that *A. malaysiensis* was the parasite used in these studies, and not *A. cantonensis*. At the time of Lim's studies the former species had not been described and the development and pathogenesis of the parasite in his monkeys resembles that of *A. malaysiensis* rather than *A. cantonensis* as shown in the present studies.

Differences in host responses, disease and pathogenesis between strains of a parasite are justification for classifying a strain of the parasite a new species. The results of exposure of monkeys to geographic strains of *A. cantonensis* only suggested that the strain of the parasite from Malaysia was different from other strains. The finding of morphologic differences between the Malaysian compared to the other geographic strains, however, provided substantial evidence of speciation (Bhaibulaya and Cross, 1971). Conclusive evidence, moreover, was demonstrated by cross-mating *A. malaysiensis* and *A. cantonensis* in hybridization experiments where it was shown that the resulting F_1 bybrid males were sterile (Cross and Bhaibulaya, 1974). Addition studies in backcrossing F_1 hybrids with parent species again showed that the F_1 hybrid males were sterile. *A. malaysiensis* is presently considered a new species and the parasite has been reported from Thailand (Bhaibulaya and Techasoponmani, 1972), Malaysia (Lim *et al.*, 1977) and Indonesia (Stafford *et al.*, 1976).

Acquired immunity is not considered to develop in humans with angiostrongyliasis since recurrence of the disease has been reported as long as one year after complete recovery from an infection (Rosen *et al.*, 1961). Monkeys, on the other hand, are able to develop immunity. In these studies monkeys previously infected with *A. malaysiensis* were able to survive challenging infection with Taiwan, Thailand and Hawaiian strains. Fruthermore, monkeys surviving infections of 500-8,000 *A. cantonensis* larvae acquired an immunity which enabled them to overcome the pathogenic effects of a challenging infection with 10,000 larvae of the Taiwan strain. All animals, however, developed eosinophilic pleocytoses which subsided 2-4 weeks postinfection. Control monkeys given 10,000 larvae died as a result of the infection.

The development of acquired immunity to *A. cantonensis* in the rodent host has been reviewed by Tharavanij (1978). In studies done by Lim *et al.* (1965) in Malaysia it was shown that rats acquired immunity after repeated infections with small numbers of worms. In the present studies it was demonstrated that rats develop a high degree of immunity to *A. cantonensis* and that the immune response was directed toward the larval stages migrating to the brain. This was based upon the finding of fewer worms in the brains of immunized animals compared to the controls. In nature it is probable that rats acquire an immunity early since mature rats are generally not heavily infected with *A. cantonensis* (Weinstein *et al.*, 1963). On Taiwan it is unusual to recover more than few worms from the lungs of wild rats and in our studies with wild rat populations the number encounted has varied from 1 to 20. On one occasion, however, 68 worms were recovered from the pulmonary vessels of an animal.

Although rats are able to develop an immunity against *A. cantonensis*, it appears that rats are not able to develop the same degree of acquired immunity to *A. malaysiensis*; the finding of 40% of the challenging infection in the brains of immunized rats compared to 54% in unimmunized rats was not considered statistically significant. Furthermore, when rats were giving an immunizing infection of *A. cantonensis* immunity which is known to develop in rats against this species appeared to have little effect on an *A. malaysiensis* challenge.

A. malaysiensis did not develop as well in rats as *A. cantonensis* in the present experiments and it is possible that this may be a factor in the development of immunity. Lim and Heyneman (1968), on the other hand, showed that low level multiple infections offered better protection than a large single dose of third-stage larvae. In these same studies they reported that the Malaysian strain did not protect rats against a large challenging infection of strains of the parasite from Thailand and Hawaii and suggested a strain specific immunity. In all likelyhood, however, the parasite used in their studies was *A. malaysiensis* and they were probably immunizing with one species and challenging with another.

Except for the earlier studies by Bhaibulaya (1968, 1974, 1975) little is known about the Australian angiostrongylid, *A. mackerrasae.* In preliminary studies reported here it appears that the pathogenesis of this species in Taiwan monkeys is similar to that found with *A. malaysiensis.* Although a small number of eosinophiles were found in the CSF of 3 animals exposed to large numbers of third-stage larvae, there was no other evidence of infections and all animals survived.

A. malaysiensis seems to be different from *A. cantonensis* both in pathogenicity and immunogenicity and further studies should be done to characterize these differences. Moreover, it is not known whether *A. malaysiensis* is associated with eosinophilic meningitis in humans in Southeast Asia. The parasite is capable of invading the CNS of monkeys and provoking a moderate meningitis with eosinophilic pleocytosis but whether clinical or subclinical disease can occur in man, remains to be determined.

SUMMARY

Studies were done to determine the pathogenicity of the Taiwan strain of *Angiostrongylus cantonensis* in the Taiwan monkey *(Macaca cyclopis)* and to determine whether differences existed in geographic strains of the parasite. The Taiwan strain of the parasite was found to kill monkeys in 7-35 days when the animals were given oral dosages of approximately 10,000 infective stage larva. At necropsy there was vascular congestion and at times hemorrhage in the CNS but little inflammation was seen in animals that died early in the infection. In animals that survived for longer periods granulomatous responses and foreign-body giant cells were found in the CNS tissue associated with dead worms. Death in the monkeys was attributed to meningitis.

Monkeys were also infected with approximately 10,000 larvae of strains of *A. cantonensis* from Thailand, Hawaii, Indonesia, Viet Nam, Philippines, Malaysia and Okinawa and infections with these strains, except that from Malaysian, was as pathogenic to monkeys as the Taiwan strain. Monkeys given the Malaysian strain, however, survived infections even when given 20,000 larvae.

The growth and development of the various geographic strains were studied in laboratory rats and worm recoveries were lower, both male and female worms were smaller, and the male spicule was smaller for the Malaysian strain compared to the other strains. The Malaysian strain was eventually described as a new species, *A. malaysiensis,* based upon

morphologic characteristics and the species validated by hibridizing experiments.

In preliminary studies the pathogenesis of *A. mackerrasae* was found *to* be similar to *A. malaysiensis* in Taiwan monkeys. Three monkeys were exposed to infection with 10,000 third-stage larvae of *A. mackerrasae* and while all demonstrated mild eosinophilic pleocytoses the animals survived the infection.

In other studies monkeys given either *A. malaysiensis* or *A. cantonensis* developed an acquired immunity that protected them against the pathogenic effects of heavy infections of *A. cantonensis*. Rats also developed a protective immunity against *A. cantonensis*, but acquired immunity in rats to *A. malaysiensis* was not pronounced. Rats immunized with *A. cantonensis* did not demonstrate a significant amount of immunity against *A. malaysiensis*. Further studies are encouraged to further characterize pathologic and immunologic differences which appear to exist between *A. cantonensis* and *A. malaysiensis* and possibly *A. mackerrasae*.

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ANGIOSTRONGYLIASIS: CLINICAL FEATURES AND HUMAN PATHOLOGY

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Eosinophilic meningoencephalitis is a clinical entity characterized by neurological manifestations related to central nervous system. (CNS) involvement associated with eosinophilic pleocytosis. The disease has a worldwide distribution, yet, the majority of cases occur in the Asian-Pacific areas, particularly in Thailand (Punyagupta *et al.*, 1970), Taiwan (Yii *et al.*, 1975), the South Pacific (Rosen *et al.*, 1967), Vietnam (Jindrak and Alicata, 1965), Malaysia (Watts, 1969; Bisseru *et al.*, 1972), Indonesia (Smit, 1962), the Philippines (Sison *et al.*, 1951) and Okinawa, Japan (Simpson *et al.*, 1970). *Angiostrongylus cantonensis* is considered the most important etiologic agent.

A. cantonensis is a neurotropic parasite that requires a period of development in the CNS of the definitive host, and will also invade the CNS of man. Although the fate of fully developed larvae leaving the nervous system of man is not known, there is evidence that some worms reach the lungs and survived for a period of time (Yii *et al.*, 1968; Sonakul, 1977). The clinical spectrum of this disease and its severity vary greatly from case to case and from one locality to the other, probably, according to the intensity of the infection and to the degree of host response. However, the clinical features are quite characteristic and leave no doubt about the diagnosis even when the causative agent cannot be identified.

This presentation will summarize the features of clinically diagnosed cases of eosinophilic meningitis presumably caused by *A. cantonensis* compared with the findings in parasitologically proven cases from other areas of Eastern Asia.

MATERIALS AND METHODS

A total of 484 cases of eosinophilic meningitis presumably caused by *A. cantonensis* studied in detail by the author and colleagues in Thailand during the 3-year period ending March 1968, and described elsewhere, (Punyagupta et al., 1970; 1975) served as a basis for analysis of the clinical features of this disease.

For comparison, 32 parasitologically proven cases of *A. cantonensis* infections from published reports and unpublished records are presented (Table 1), i.e., 19 cases from Thailand, 10 cases from Taiwan and one case each from Malaysia, Indonesia and North Vietnam.

Postmortem studies of the parasitologically proven cases are summarized in the section on pathological findings.

Table 1

List of 32 parasitologically proven cases of human angiostrongyliasis used in the clinical evaluation.

Case No.	Sex	Age	Country	Worms recovered from	Authors
1	F	28	Thailand	Brain	Tangchai *et al.*, 1967
2	F	48	Thailand	Brain	Nye *et al.*, 1970
3	M	21	Thailand	Brain	Chitanondh *et al.*, Personal communication, 1964
4	M	34	Thailand	Eye-anterior chamber	Prommindaroj *et al.*, 1962
5	M	22	Thailand	Eye-anterior chamber	Ketsuwan *et al.*, 1966
6	M	21	Thailand	Eye-anterior chamber	Ketsuwan *et al.*, 1965
7	M	36	Thailand	Eye-posterior chamber	Kanchanaranya *et al.* 1972
8	M	34	Thailand	Eye-posterior chamber	Kanchanaranya *et al.*, 1971
9	M	29	Thailand	CSF	Bunnag *et al.*, 1969
10	F	12	Thailand	Brain	Tangchai *et al.*, 1966, Per. Comm.
11	F	29	Thailand	Brain	Hongladarom *et al.*, 1963
12	F	40	Thailand	CSF, Eye-posterior chamber	Laopatarakasem *et al.*, 1975
13	M	34	Thailand	Brain	Indaravasu, 1975, Per. Comm.
14	M	34	Thailand	CSF, Brain, Lungs	Rodprasert *et al.*, 1974; Sonakul 1978
15	M	21	Thailand	Brain, CSF	Sonakul, 1978 (cases 15–17)
16	F	26	Thailand	Brain	
17	F	3½	Thailand	Brain, Cord, CSF	
18	F	43	Thailand	Cord T 5-6	Pairojkul *et al.*, 1977
19	M	21	Thailand	CSF	Nitidandhaprabhas *et al.*, 1975
20	M	27	Malaysia	CSF	Bisseru *et al.*, 1972
21	F	23	Indonesia	Eye-anterior chamber	Sunardi *et al.*, 1977
22	F	18	Vietnam	Brain	Jindrak and Alicata, 1965
23	M	15	Taiwan	CSF	Nomura and Lin, 1945
24	F	5	Taiwan	Brain, Lungs, Cord	Yii, 1976 (cases 24–32)
25	M	7	Taiwan	CSF	
26	M	5	Taiwan	CSF	
27	F	4	Taiwan	CSF	
28	M	8	Taiwan	CSF	
29	F	3	Taiwan	CSF	
30	M	3	Taiwan	CSF	
31	M	19	Taiwan	CSF	
32	F	7	Taiwan	CSF	

CLINICAL FEATURES

Age and sex. In our study of 484 cases, the youngest patient was 2 years old and the oldest 65. In Thailand the disease is more prevalent in adults 20-34 years of age. In 32 proven cases the age ranged from 3 to 48 years. In Taiwan, however, the disease is more common in children, with the average age of 7.6 years and 70% were under 10. The discrepancy of the age incidence is probably due to the different methods of infection in different geographic areas.

In Thailand males were 2.6 times more commonly infected than females, but in Taiwan 1.2 times more females than males were infected. However, among the 32 proven cases males were infected 1.3 times more than females. This probably represents the difference in the exposure to the common infection source.

Incubation period. Out of 484 cases studied in Thailand 145 were members of 37 groups of two or more persons who had shared the same dishes of raw or undercooked *Pila* spp. snails which have been shown to be the most important source of infection. Therefore, the incubation period could be identified. The shortest interval was 3 days, the longest 36 days and the average 16 days. Only 9 of the 145 cases had incubation periods longer than 30 days.

In the 32 proven cases the incubation periods were known in only 6 cases from Thailand and the periods varied from 1-20 days with the average of 8.3 days.

Initial systemic symptoms. Among the proven cases 3 patients from Thailand experienced vomiting within few hours after ingestion of *Pila* spp. snails. Abdominal pain and diarrhea were noted in one case. Early abdominal symptoms were also observed in some of the 484 cases. This may represent the host reaction to the infected food, such as raw or undercooked snails or possibly to the gastrointestinal invasion by *A. cantonensis* larvae. Five cases from Taiwan also experience gastrointestinal symptom in the initial stage of the disease, but this was probably the result of cerebral involvement. In addition, maculopapula or urticarial skin rashes have been observed soon after ingestion of snails in 2 proven cases and in some unconfirmed cases. The rashes lasted for a few days and may have been due to allergic reactions to the food.

These early systemic symptoms occurred in only a few cases after which the patients became asymptomatic for a period of time prior to the development of symptoms secondary to the CNS involvement.

Chief complaint. Headache was the chief symptom in 96% of cases. The remainder came to seek medical care because of convulsions, weakness of the extremities, paraesthesia, vomiting, facial paralysis, stiffness of the neck and fever. In all of the proven eye cases only one patient experienced preceding headache; the rest presented eye symptoms. About 77% of cases came to the hospital within 10 days after having the first symptom. In Thailand, the onset of headache was rather gradual but it was considered an abrupt onset in 78% of cases from Taiwan where more cases were found in children. Headache was usually described as severe, intermittent, head-breaking and unbearable. Each attack of headache lasted from a few minutes to few hours. The common site of headache was occipital and bitemporal. Most cases had never experienced such severe headache in the past. Ninety-nine percent of meningitis cases experienced severe headache, yet, in most proven eye cases, headache had never been noticed in spite of the fact that the lumbar puncture revealed eosinophilic pleocytosis (Kanchanaranya and Punyagupta, 1971).

Clinical manifestations relating to CNS involvement. Nausea, vomiting and neck stiffness were noted in about half of the cases and these symptoms subsided soon after the spinal tapping and analgesic administration. Physical examination revealed stiffness of the neck and positive Kernig's sign in only 15% and 6% in Thai patients and 57% and 44% of the Taiwanese, respectively. It was noted more frequently in confirmed cases.

Sensorium impairment of varying degrees was observed in 5% of the clinical cases in Thailand but in a much higher degree (82%) in cases from Taiwan. In 19 proven meningitis cases lethargy and coma were noted in 17, but it was not found in 6 proven eye cases. This symptom probably represented the severe degree of cerebral involvement. Only one out of the 484 clinical cases from Thailand developed deep coma but recovered fully compared to 14 of 32 parasitologically confirmed cases where none of the patients survived. From Taiwan, 11 out of 125 patients developed coma and 4 died; the remainder experienced a long semi-comatose stage.

In Thailand, 2 cases were admitted to a psychiatric ward because of frank psychosis; they were found to have eosinophilic meningitis. The psychosis disappeared soon after the meningitis had subsided. Two mental institutions in Thailand were surveyed and a few cases of eosinophilic meningitis were found. The patients ingested raw snails and slugs and were considered mentally retarded. Convulsion occurred in 4% and 3% of the clinical cases from Thailand and Taiwan, respectively. Urinary incontinence and retention were observed in some severe cases with sensorium impairment.

Only 3 cranial nerves were commonly involved, namely, the optic nerve as shown by the visual impairment in 16% of the cases in Thailand, facial nerve paralysis (4% and 1% in Thailand and Taiwan, respectively), and lateral abducens nerve paralysis (3% and 8% in Thailand and Taiwan, respectively). Similar findings were noted in proven cases. The cranial nerves impairment were usually unilateral but at times bilateral.

Peripheral nerve involvement as demonstrated by the symptoms of paraesthesia of the small areas in the trunk or extremities was observed in 37% of clinical cases and in 8 proven cases. This symptom may persist for a few weeks or months.

Generalized weakness of the extremities has been observed in 1% of 484 clinical cases in Thailand. Seven out of 125 Taiwan cases (6%) experienced definitive flaccid paralysis of the extremities associated with coma and 19 other cases experienced motor weakness of the extremities. In 32 proven cases generalized motor weakness was found in 9. This may have been due to the spinal cord involvement by the worms as shown by the finding of worms in the spinal cord in 2 postmortems (Yii *et al.*, 1968; Pairojkul *et al.*, 1977).

Unilateral, or less frequent bilateral, swelling of the eye lids were noted in 1% of the clinical cases from Thailand. The symptoms may represent the periocular migration of the worms following the brain migration as observed similarly in experimented animals (Kanchanaranya and Punyagupta, 1971).

Malaise and anorexia were constitutional symptoms observed in some cases during the early acute stage.

Low fever was noted in 29% of clinical cases from Thailand and only 4% had temperature of over 38°C. This is in contrast to the other forms of meningitis. However, higher fever was more common in the comatose patients of the parasitologically proven cases. Experiences in Taiwan also show that fever was more predominant in children and it lasted for several weeks.

Ocular manifestations. Ocular involvement by *A. cantonensis* is a wellknown in both human and experimental animals (Prommindaroj *et al.*, 1962; Ketsuwan and Pradatsundarasar, 1965, 1966; Kanchanaranya and Punyagupta, 1971; Kanchanaranya *et al.*, 1972; Punyagupta *et al.*, 1975). In Thailand *A. cantonensis* was first found in the anterior chamber of a patient prior to recognition as a cause of eosinophilic meningitis (Prommindaroj *et al.*, 1962). In 484 proven cases 78 cases or 16% were proved to have failing vision of varying degrees with bilateral in 47 cases and unilateral in 31 cases; diplopia and abnormal visual fields accounted for 1% each. Examination of the fundus revealed abnormalities in 12% of the cases. Permanent blindness is not uncommon.

In 7 proven eye cases, 5 males and 1 female from Thailand and 1 female from Indonesia (Sunardi *et al.*, 1977), failing of vision was the main complaint while one case was admitted because of a worm moving in the anterior chamber of the eye. Only a single fifth-stage larva or a young adult *A. cantonensis* was recovered from each case; from the anterior chamber in 4 cases and from the posterior chamber in 3 other cases. Hemorrhages and detachment of the retina are serious complications of retinal angiostrongyliasis.

The best way to visualize the small moving worms in the posterior chamber is by using the slit lamp. Only 2 cases were admitted with symptoms of headache and stiffness of the neck suggesting eosinophilic meningitis. Lumbar puncture was done in only 3 eye cases and all showed eosinophilic pleocytosis. In one case (Laopatarakasem *et al.*, 1975) *A. cantonensis* was seen in the retina and 6 others worms were recovered from the spinal fluid on the same day. It is true that in most eye cases the meningitis symptoms are minimal and lumbar punctures are not considered. The size of *A. cantonensis* recovered from the eyes were 8.5-18.86 mm in length, as large as or larger than those recovered from spinal fluid or the brain at autopsy.

Pulmonary symptoms. Among 484 clinical cases none experienced definitive symptoms nor signs of pulmonary involvement. However, 3 parasitologically proven cases experienced productive cough and audible rales over the lungs suggestive of pneumonitis and the chest X-rays confirmed abnormalities. In 2 postmortems, one from Taiwan (Yii *et al.*, 1968) and another from Thailand (Rodprasert *et al.*, 1974; Sonakul, 1978), adult and larvae *A. cantonensis* were found in the lung tissue. These findings strongly suggested that *A. cantonensis* can migrate to the lungs after leaving the brain and produce some abnormal pulmonary manifestations. In the early stage of illnesses many patients from Taiwan (Yii, 1976) experienced intermittent dry cough and in some cases associated sneezing and rhinorrhea.

LABORATORY FINDINGS

Blood. Leucocytosis was observed in 56% of cases and associated with eosinophilia (over 10%) in 73% of cases. The initial increase in leucocyte counts occurred on the 17th day after infection while eosinophilia reached the peak on the 30th day. Leucocytosis and eosinophilia of varying degrees were noted throughout a period of 3 months. Similar findings were found in proven cases.

Cerebrospinal fluids (CSF). Elevation of the initial CSF pressure above 200 mm of water was observed in 54% of clinical cases and in many cases higher than 500 mm of water was experienced. The fluid was grossly opalescent or turbid in 88% of clinical cases. It had the appearance of water after it has been used to wash milled rice. Slight xanthochromia was noted in only one case from Thailand and 2.4% of the cases from Taiwan, and microscopic red blood cells were seen occasionally.

A. cantonensis larvae were recovered from the spinal fluid in 16 of 32 cases on 24 occasions, that is, in 9 children from Taiwan, and 1 from Thailand, in 5 adults from Thailand and one adult from Malaysia. In Taiwan where infections are considered much heavier than elsewhere, *A. cantonensis* larvae were recovered in the CSF in 8 out of 88 children under 10 years of age and only one out of 37 patients over 10 years of age. This suggests a greater chance of recovering worms from the CSF in small children and is probably due to the smaller subarachnoid space and less amount of spinal fluid.

The worms, most of the time alive and motile fifth-stage or young adults, were recovered, at the most, 3 times from one patient. In many instances the larvae were obtained by using ordinary 20-gauge lumbar puncture needles without aspiration. However, using a larger bore needle and applying negative pressure by a syringe may increase the chance of recovering the larvae particularly at the later stages of the illness. Some of the larvae recovered were very small, therefore, one must examine the fluid carefully against the light to visualize the moving larvae. In one instance, eosinophiles were found to be aggregated around the cuticles of a dead worm. Worms were generally recovered from the CSF between the 5th and 19th days after the occurrence of the first symptom. The reason for not recovering worms at later dates was probably because of the greater size of the worms. In a case from Thailand (Bunnag *et al.*, 1969) where the exact date of infection was known, the worms were recovered from the fluid on the 8th, 11th and 18th days after infection.

Eosinophiles as well as polymorphs and lymphocytes in the spinal fluid may be easily recognized by examining the fresh, unfixed and unstained fluid under 40 x magnification using a dim light. Occasionally, larger cells believed to be ependymal cells or plasmacytes were seen. Eosinophiles are easily disrupted during the process of staining. Modified Giemsa's stain yielded better result than ordinary Wright's stain.

The initial number of white cells in the CSF was in the range of 500 to 2,000 per cu mm, with 26% below 500 per cu mm and about 20% over 2,000 per mm. In the proven cases the cell counts were 190-4,345 per cu mm.

As for eosinophilia of the CSF, the initial examination revealed eosinophilia of lower than 10% in 4% of clinical cases, yet, all showed eosinophilia of above 10% in subsequent examinations and some as high as 90% and over. In the proven cases, eosinophilic pleocytoses of 15-98% were found.

In a group of 25 cases of whom definite dates of infections were certain, eosinophilic pleocytoses were recognized on the 12th day after infection with a peak ranging between 25 and 30 days. After one month eosinophilia dropped and in some cases rose again to another peak between the 75th to 80th days before dropping to a very low level on about the 90th day. The second rise in eosinophilia is believed to be due to dead larvae in the CNS at the later stages of the disease.

About 68% of the cases showed increases in protein contents of the spinal fluid of above 50 mg per 100 ml but the sugar contents were low in only 9% of cases. The highest levels of protein content were found around the 25th and 40th days. In a study on the immunoglobulin of the CSF (Tungkanak *et al.*, 1972) IgG and IgA were found to be significantly higher than that of the controls and proportionally higher than the corresponding serum immunoglobulins. These findings indicate that the immunoglobulin were synthesized locally in the CNS in response to the larval migration.

Stools, urines and other biochemical tests were insignificant.

Skull X-rays and the chest X-rays of the clinical cases revealed no abnormalities. However, the chest X-rays in 3 proven cases experiencing lung symptoms, showed abnormalities suggesting pneumonitis or congestion.

Electroencephalograms done in some severe cases revealed nonfocal abnormal slow dysrhythmia.

Serological diagnosis is not helpful because of the lack of specificity of the tests.

DIAGNOSIS

In endemic areas, eosinophilic meningitis caused by *A. cantonensis* should be considered in patients who experience any of the following clinical features:

1. Acute severe headache associated with or without low fever.
2. Symptoms and signs of meningitis or meningoencephalitis particularly with low fever.
3. Cranial nerves involvement, namely, facial palsy, lateral rectus paralysis associated with severe headache.
4. Ocular manifestations, namely failing of vision or diplopia of one or both sides with or without headache.
5. Psychosis or sensorium impairment associated with severe headache.

An important clue in the diagnosis is the history of ingestion of suspected food, such as raw or undercooked *Pila* spp. snails or the giant African snail, *Achatina fulica*, within a period of one month prior to the first symptom. Spinal tapping is the most important procedure in the diagnosis.

Based on the clinical features alone the disease may be confused with the following conditions:

1. Migraine headache or brain tumor because of severe headache.
2. Psychoneurosis because of strong complaint of headache without obvious neurological abnormalities.
3. Purulent meningitis because eosinophilic pleocytosis is misinterpreted as neutrophilia.

4. Tuberculous or viral meningoencephalitis because the majority of cells in the spinal fluid are lymphocytes.

5. Encephalitis because of sensorium impairment in some severe cases.

These conditions may be easily excluded by examining CSF for eosinophiles.

In Thailand where another form of *eosinophilic meningoencephalitis* caused by a different parasite, *Gnathostoma spinigerum*, exists, one may differentiate the two diseases on clinical grounds with a reasonable degree of certainty (Punyagupta *et al.*, 1968a; 1968b). Patients with eosinophilic myeloencephalitis caused by *G. spinigerum* usually present with the following complaints:

1. Paralysis of the extremities characterized by transverse or ascending myelitis following the symptom of nerve root pain (radiculitis) of the involved extremities or of the trunk.

2. Comatose patients particularly in younger individuals who may or may not experience paralysis of the extremities. The spinal fluids are bloody or xanthochromic with eosinophilic pleocytosis.

Based on the clinical and epidemiological information the presumptive diagnosis of the cerebrospinal angiostrongyliasis can be made. Yet, the definitive diagnosis can only be made by findings of the worms in the CSF, in the eyes or at necropsy.

TREATMENT AND COURSE OF THE DISEASES

The disease is self-limiting and usually lasts for 4-6 weeks. In mild and moderately severe cases, analgesics are used but the results are not encouraging. The severe headache usually subsides dramatically, yet, temporarily after the spinal tapping and removal of about 10 ml of the fluid. However, the headache may reappear within a few days. Repeated spinal tapping (2-4 times) is usually necessary in most cases. In about 40% of cases, headache persists longer than one month.

Corticosteroids in the form of oral prednisolone at the dosage of 30-60 mg daily have been given in combination with analgesics in 96 cases and the results were statistically insignificant from the controls. Considering the pathogenesis of this disease, immunologic reaction is probably one of the main factors. Therefore, in a very severe case with cranial nerve involvement, such as visual impairment, systemic corticosteroid therapy may be helpful. Nevertheless, intensive general and neurological care are the most important methods in saving lives in critical cases.

Among the clinical cases 41% were mild and not admitted to the hospital. For those admitted 41% of the cases were discharged from the hospital within the first 5 days with improvement and only 6% stayed in the hospital longer than one month. Some cases were readmitted because of recurrence of headache on the 2nd and 3rd weeks. The lumbar puncture showed an increase in the number of cells compared to previous examinations.

Stiffness of the neck, nausea and vomiting were the first symptom to disappear, usually within a few days. Visual abnormalities improved slowly after a few weeks. Paraesthesia took a much longer time to disappear. The cranial nerve involvement was the last to recover and permanent residual defects were not infrequently seen.

The recurrence of the disease in the same patient a few years apart had been verified in only 2 cases in our study and the epidemiological data indicated these to be due to reinfections. The clinical severity was similar to the first infection.

In Thailand the mortality rate is very low. Only one out of 484 clinical cases died with the symptoms of cerebral depression. In Taiwan (Yii, 1976) 4 out of 125 cases died, but in South Pacific (Rosen *et al.*, 1961), there were no fatalities out of several hundred cases. It should be mentioned that all eye cases survived, and 12 out of 16 cases where the larvae were recovered from the CSF also survived. In 32 parasitologically confirmed cases, 13 died. The severity of the disease is probably related to the number of larvae ingested. In 13 proven cases where the patients died, all had cerebral depression with 3 cases also having pulmonary symptoms. Death occurred as early as the 14th day and as long as 80 days after the first symptoms, but the majority died between 2-4 weeks.

In eye cases, surgical removal of the worm from the anterior chamber is simple but to remove the worm from the posterior chamber requires cryosurgery and an experienced surgeon. The technics have been described (Kanchanaranya *et al.*, 1972).

PATHOLOGY IN MAN

The neuropathology of human angiostrongyliasis has been well described by Rosen *et al.* (1962), Jindrak and Alicata (1965), Tangchai *et al.* (1967), Nye *et al.* (1970), and Sonakul (1978). Other, yet unpublished findings at autopsy have been presented by Hongladarom (personal communication, 1963), Indaravasu (personal communication, 1975) and Pairojkul *et al.* (1977). The neuropathology in human cases is summarized as follows:

Gross pathology. Examinations of the external surfaces of the brains and spinal cords generally were unremarkable. In some cases evidence of brain congestion on the pressure cone may be recognized. Leptomeninges of the basal portion of the brain may show some thickening. Gross hemorrhages were most unusual. In one autopsy in our series (Nye *et al.*, 1970) an area of gross hemorrhage of about 1 x 2 cm was seen on the coronal sections and a very large *A. cantonensis*, 18.3 mm in length and 0.41 mm in width, was recovered near the area of hemorrhages.

Larvae of *A. cantonensis* are often seen on the surface of the brain or spinal cord, but most of the time they were visualized only in microscopic sections. The worms were recovered from the normal saline in which the spinal cord and meninges were placed (Yii, 1976). Pairojkul *et al.* (1977) recently described finding a living *A. cantonensis* in the subarachnoid space at the thoracic 5-6 levels of the spinal cord.

Microscopic findings. In any postmortem of a suspected case of *A. cantonensis* infection, more than the routine 10 blocks of the different parts of the brain and spinal cord should be made to increase the chance of recovery of the worms. The cross-sections of the worms are usually distinctive and easily recognized under microscopic low power scanning of the sections. The diameter of the cross-section of the worms range between 30-90 µm. The largest section we have found was 300 µm. If serial sections of the positive blocks were made and the graphic reconstruction of the worm was done, the characteristic features of *A. cantonensis* can be achieved. Larger size worms can be removed from the brain tissue under dissecting microscopy. The number of the worms recovered from autopsies has varied from few larvae out of blocks of brain tissue to hundreds. Sonakul (1978), in Thailand, found 72 worms, from a postmortem and Yii (1976) on Taiwan recovered more than 650 young adults from one autopsy.

A. cantonensis larvae were easily recognized in the brain tissue, in the meninges and sometimes in the blood vessels or perivascular spaces and the worms may be alive or dead at the time of necropsy. Cellular reaction was minimal around the living worms and more pronounced around the dead worms. Granulomatous inflammatory reactions were seen to be composed of mononuclear cells, particularly, lymphocytes, plasma cells, macrophages and of eosinophiles. Polymorphonuclear cells were predominant in some granulomatous areas and Charcot-Leyden crystals in other areas. Cellular reactions were observed not only around the worms but also along the meninges and intracerebral vessels.

One of the most characteristic features of the neuropathology was the finding of multiple microcavities or tracks representing passages of migrating worms. Microscopic findings of the tracks showed disruption of the brain tissue, debris, gitter cells, cellular infiltrations and at times evidence of microscopic hemorrhage. The non-hemorrhagic tracks are usually smaller than 150 µm differing from larger tracks caused by *G. spinigerum*. One striking microscopic feature was the vascular dilatation, both arterial and venous, in the subarachnoid space. The nerve cells in the adjacent areas to the worms or tracks may exhibit central chromatolysis and cytoplasmic axonal swelling. Similar pathological findings may be seen in areas of the spinal cord.

Lungs were the only other organ found to be involved by this parasite. Yii *et al.* (1968) made the first report of finding mature *A. cantonensis* in the lungs of a 5-year-old girl who died of eosinophilic meningitis on Taiwan. Subsequently, Sonakul (1978) found 2 degenerated *A. cantonensis* in the pulmonary artery of a 34-year-old Thai woman who died of the disease 19 days after experiencing the first symptom. Pulmonary hemorrhages and terminal bronchopneumonia were also seen in the sections.

In spite of the fact that *A. cantonensis* has been recovered from human eyes, there are no description of the pathology associated with eye infections in man.

CONCLUSION

A. cantonensis is considered the most important cause of eosinophilic meningitis in man in Asia. In Thailand, as well as in Taiwan, hundreds of cases are recognized each year. The clinical features of the disease are well described so that reliable diagnoses can easily be made. Although the disease is self-limiting and the majority of the cases are considered mild, fatalities are not infrequent. There is no effective specific therapy for the infections or the disease but intensive neurological, cardiopulmonary and good general care during the comatose stage will save the lives of seriously ill patients. The neuropathology of the disease is so unique that it leaves no doubt about the diagnosis at necropsy. Pathologists in endemic areas should be attentive to pathological changes which may occur in other organs, namely, the heart, lungs and the eyes.

Considering the epidemiology, morbidity and mortality, angiostrongyliasis should be listed as one of the more important infectious diseases in the Asian-Pacific area. In spite of the fact that the infection can be prevented, we continue to find as many cases today as in 1965 when we first began to study this newly recognized disease. Sincere and concerted efforts by medical and public health agencies will be required to effectively control this parasitic disease.

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IMMUNOLOGY OF ANGIOSTRONGYLIASIS

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ACQUIRED IMMUNITY

Evidence suggesting the existence of specific acquired resistance to *Angiostrongylus cantonensis* infections in mammalian hosts is the recovery of a low number of parasites from wild rats in endemic areas (Weinstein *et al.*, 1963; Alicata and Jindrak, 1970). On the other hand, immunity in man is considered less complete, since the disease is known to recur within a few months after a previous attack (Rosen *et al.*, 1961; Alicata and Jindrak, 1970). In rats infected experimentally with a low dose of infective third-stage larvae of *A. cantonensis* and then challenged with a larger number of infective larvae, a certain degree of resistance was observed (Lim *et al.*, 1965; Heyneman and Lim, 1965; 1967). Furthermore wild rats, *Rattus jalorensis,* found naturally infected with *A. cantonensis* had a reduced worm recovery rate upon challenge with 300 larvae (Lim *et al.*, 1965). Similar findings were obtained in laboratory reared white rats receiving 1-3 feedings at monthly intervals of 5-15 infective larvae and then challenge with 1000 infective larvae (Heyneman and Lim, 1965). Such protection was not absolute, however, since the immunized rats eventually succumbed to the infection, even though some survived well over 100 days (Heyneman and Lim, 1965). Protection was thought to be strain specific, since protective immunity failed to develop in rats immunized with one strain and challenged with others (Lim and Heyneman, 1969; Heyneman and Lim, 1967; Lim, 1968). For example, rats immunized with low doses of a Malayan strain were not protected against Hawaiian, or Thailand strains (Lim and Heyneman, 1969).

In view of the fact that the work suggesting strain specificity of immunity had been carried out mostly in Malaysia before the recognition of a new species *A. malaysiensis* (Bhaibulaya and Cross, 1972), which was prevalent in Peninsular Malaysia (Lim, 1975), it was highly likely that the so-called "Malayan strain" of *A. cantonensis* used in these studies was in fact *A. malaysiensis.* It follows that reported demonstration of strain specific immunity would rather be demonstration of species specific immunity in *Angiostrongylus* infection.

Another way of achieving substantial protective immunity against lethal challenge of infective larvae is by immunization with irradiated third-stage larvae. Lee (1969) showed that rats receiving 2 feedings of 200 third-stage *A. cantonensis* larvae, irradiated with 40 Kr, developed significant resistance to further challenge with a lethal dose of unirradiated infective third-stage larvae. In contrast, attempted immunizations of rats with non-living antigens obtained from different stages of *A. cantonensis* such as dead third-stage, dead first-stage, and extracts from adult worms or from larval stages from the brain, failed to confer protection against the subsequent challenge with a lethal dose of infective larvae (Lungdhara, 1974). It appears then that immunity to the parasite is induced by factors associated with living larvae, or adult worms. This protective immunity could be a "premunition" which, according to Sergent (1963), refers to the resistance of the host in a state of latent infection against a superinfection by a parasite of the same species. According to this definition, it is not clear whether the premunition state needs the presence of adult worms or the mere presence of the larval stages is sufficient. There is some evidence indicating that the presence of adult worms may not be required, since it was shown that in rats infected with irradiated larvae, adult worms seldom develop, and yet these rats developed a considerable degree of immunity (Lee, 1969). Radiation is known to interfere with the development of *A. cantonensis* and causes most of the young adult worms to lose ability to migrate to the lung of the definitive host therefore eliminating the chance to mature to the adult stage.

The real factor(s) associated with protective immunity induced by live parasites are not known. It is likely that such factor(s) are derived from the excretory-secretory product (ES antigen). Uahkowithchai *et al.* (1977) prepared ES antigen from adult male and female worms by incubating the parasites in NCTC 109 medium at 37°C in 5% CO_2 atmosphere for 24 hours using 0.1 ml of the medium per worm. Rats were given 5 injections (Schedule I) or 6 injections (Schedule II) of the culture fluid at weekly interval with graded increment of the fluid volume from 0.5 to 2 ml, followed by challenge with 150 or 500 infective third-stage larvae either at 14 days after the last immunizing dose (Schedule I) or immediately after the 5th injections to be followed by the 6th injections a week later (Schedule II). The results are shown in Table 1. It was found that rats immunized with the female ES antigen had significantly lower mortality rate and a longer mean survival period than the control group, especially when the challenge dose was 150 infective larvae. Immunization with the male ES antigen had no protective effect. It is not known whether such findings reflects the quantitative difference of the amount of the ES antigen used or whether it indicates that the protective ES antigen(s) are associated with the female reproductive system (Uahkowithchai *et al.*, 1977). The protection invoked by the female ES antigen is partly mediated by the humoral antibody, since in a preliminary test the protective effect could be transferred passively by rabbit anti-ES serum (Uahkowithchai *et al.*, 1977).

The mechanisms whereby immunity operates against *Angiostrongylus* infections are not clearly understood. Examination of parasites in immune animals showed that the worms recovered were relatively smaller in number than those in the control group (Heyneman and Lim, 1965; Lee, 1969; Unhkowithchai *et al.*, 1977). In rats experimentally infected with low dose of infective larvae, and those infected with larvae irradiated with 40 Kr, the worms were found dead in large numbers (Heyneman and Lim, 1965; Lee, 1969) and those alive were stunted in development. Stunting effect was also demonstrated in rats immunized with the female ES antigen (Uahkowithchai *et al.*, 1977). The mechanisms leading to death of the parasite or responsible for retarded development are not known. Factors related to the humoral immune response the cell mediated immune response or the antibody dependent cell mediated cytotoxicity (ADCC) may be involved.

Table 1

Effect of immunization with ES antigens from adult *Angiostrongylus cantonensis* on the course of experimental infection of rats with third-stage larvae

Pre-treatment	Method of immunization	Number of rats	Challenging does (Number larvae)	Mortality rate	Survival period (days ± S.E.)	Total recovery (%)
Male ES antigen	schedule I	5	500	100	20 ± 0.4 •	32 ± 3.8
	schedule II	5	150	100	25 ± 0.5	36 ± 4.1
Female ES antigen	schedule I	4	500	100	29 ± 0.9	33 ± 4.4
	schedule II	5	500	100	38 ± 2.7	14 ±1.6
NCTC 109 medium	schedule I	12	500	100	23 ± 0.4	56 ± 1.5
	schedule II	5	150	100	25 ± 0.6	44 ± 3.2

Data taken from Uahkowithchai *et al. Southeast Asian J. Trop. Med. Pub. Hlth.* 8: 486,1977.

HUMORAL IMMUNE RESPONSE

The presence of antibodies in the immune rats or rats experimentally infected with the infective larvae has been amply documented. The antibodies were detected by the indirect haemagglutination test (Kamiya and Tanaka, 1969; Chen and Suzuki, 1974; Yoshimura and Yamagishi, 1976), immunodiffusion and immunoelectrophoresis (Mishra and Benex, 1970), *complement fixation test* (Mishra and Benex, 1970), immunofluorescent test (Ishii and Kamiya, 1973; Chen and Suzuki, 1974) and latex agglutination test (Mishra *et al.*, 1970). How humoral antibodies interfere with growth and development of the angiostrongylids are not known. One possible mechanism is that the antiboby causes microprecipitation around the orifices thus interfering with normal metabolism of the worm (Bhaibulaya, 1971). Such Sarles (1938) type reaction was found to be genus specific, since precipitates occurred around the orifices of fifth-stage larvae of *A. cantonensis* or *A. mackerrasae* in the presence of homologous and heterologous sera (Bhaibulaya, 1971). Furthermore, *in vitro* exposure for 2 hours of the adult female angiostrongylids to the sera obtained from rats at various times after infection with 50-200 infective larvae showed a significant inhibition of the oxygen uptake (Kanjanabutara, 1976). The suppression of the oxygen uptake was evident as early as 7 days after infection and remained so for the period of 3 months. Heat inactivation of the sera at 56°C for one-half hour abolished the oxygen consumption inhibitory effect which indicated that complement was required for this reaction (Kanjanabutara, 1976).

The time sequence of humoral immune response have been extensively studied. Kamiya and Tanaka (1969) studied the production of haemagglutinating antibodies in rats infected orally with 20-60 infective larvae. The antigen was Tris-buffered saline (pH 7.4) extract of adult worms. A significant titer (1:32) was obtained in 15 out of 17 rats during 6-10 weeks and all 17 rats after 10 weeks were positive with higher titers. Similar finding was observed by Yoshimura *et al.* (1976) who showed that rats infected orally with an average number of 77 infective third-stage larvae produced a progressive increase in haemagglutinating antibody beginning from 6 weeks after infection (Yoshimura and Soulsby, 1976). Slightly different result of the time of emergence of significant haemagglutinating antibody in the sera of rats infected with the Taiwan strain of infective third-stage larvae were reported by Chen and Suzuki (1974), in which a mean titer of a little more than 1:160 was detected in the third week (rather than 6-10 weeks) after infection with 100 and 200 infective larvae. The reason for this decrepancy is not known, but it could be due to the strain difference of the parasites or the rats used in the experiment.

The appearance of detectable antibody coincided with the appearance of larvae in the feces (Kamiya and Tanaka, 1969), which suggested that antigenic stimulation was released mainly by adult worms. Such antigenic factors would be associated mostly with the female worms, since Jacobs *et al.* (1965) showed that sera from infected rats interacted in the haemagglutination test with red cells sensitised with the culture fluid of adult female but not that of adult male worms. Further work by Kamiya *et al.* (1972) showed that rats transplanted intraperitoneally with adult female worms produced detectable haemagglutinating antibody at one week after transfer whereas those transplanted with adult male worm did not produce antibody until the 6th week after transplantation. This study lends additional support to the contention of Jacobs *et al.* (1965) that the haemagglutinating antibody was directed against the antigens associated with maturity and fecundity of the adult females. In contrast to the haemagglutinating antibody the appearance of the antibody in the latex agglutination test is much earlier. According to Mishra *et al.* (1970) the latex agglutinating antibody was positive with a titer of 1:2 as early as 5 days after infection followed by a progressive rise with a titer of 1:64 in 10-17 days. This augmentation corresponded to the phase of the 4th moulting and migrating of the parasites to the surface of the brain (Mishra *et al.*, 1970).

The precipitin antibodies are in general produced in experimental rats. Mishra and Benex (1970) showed the presence of 2 precipitating lines located near to the antigen well in the immunoelectrophoresis test as early as 6 days after infection with a rise in staining intensity up to 40 days of observation.

Kamiya (1975) performed the immunoelectrophoresis test sequentially in 5 rats infected with 100 third-stage larvae. In general 3 bands were observed. The band on the cathode side near to the antigen well was demonstrated as early as 1 week after infection followed by 2 other bands one at the cathode side, and the other at the anode side some 2-6 weeks later. Yoshimura *et al.* (1976) found as many as 5 immunoelectrophoretic bands 6 days after infection with a mean of 77 third-stage larvae of the Hawaiian strains of *A. cantonensis.* Thereafter the number of bands varied between 5-8 up to 63 days after infection.

The immunofluorescent antibody titer in sera of rats at various times after infection with third-stage larvae was first reported by Mishra and Benex (1970). The antibody was detectable as early as 4 days after infection. The reaction was strongly positive at 20 days after infection and remained so for 11 months in chronically infected animals (Mishra and Benex, 1970). Chen and Suzuki (1974) showed that sera of rats infected with 100 larvae were positive with an average titer of 1:40 at the 10th day after infection, followed by a gradual increase to a maximum titer of around 1:640 at day 50. Similar finding was obtained with a larger infective dose (i.e. 200 larvae), except that the titer on day 10 was lower. It should be noted that antibody binding sites of *A. cantonensis* were internal tissues, muscle in both larval and adult stages, but no specific reaction was observed in the cuticle (Ishii and Kamiya, 1973; Chen and Suzuki, 1974).

Production of reaginic antibody in rats and rabbits infected with *A. cantonensis* larvae as measured by the passive cutaneous anaphylatic (PCA) test in homologous animals was reported by Yoshimura and Yamagishi (1976). In 2 out of 3 rabbits, the reaginic antibody was detected 9 weeks after primary infection and persisted at very low titers thereafter. With secondary infection, the antibody was detected sooner (i.e. 5 weeks) and the PCA titer increased markedly. In rats, the peak reaginic response was observed 5 weeks after primary infection. In contrast to that found in the rabbit, the antibody production was only transient and no anamnestic reaction was induced by reinfection.

CELL-MEDIATED IMMUNE RESPONSE (CMIR)

Rat. The cell-mediated immune response to angiostrongyliasis has been demonstrated by 2 different methods, i.e. the blast transformation of lymphocytes in the presence of the antigen and the migration inhibatory factor (MIF) using either somatic and ES antigen. With the blast transformation test, Yoshimura and Soulsby (1976) showed that lymphocytes (2×10^6 cells in 1.8 ml) from cervical lymph nodes of rats infected with a mean number of 77 infective larvae of Hawaiian strain underwent significant transformation upon exposure in vitro to 50 µg of the crude extract of adult worms. The response was observed during 1-4 weeks postinfection which was the time when the larvae were developing in the brain tissue. Thereafter (5-17 weeks postinfection), the blastogenic response was apparently high, but statistically this was not different from that of the control. It is possible that the number of tests used in these experiment was not sufficiently large to be of significance, and it is therefore recommended that additional studies be done to determine the duration of blastogenic response after infection. In subsequent work by Yoshimura and co-workers (1976) the direct and the indirect MIF tests in agarose droplet were used. In the direct test, lymphoid cells from lymph nodes were mixed with guinea pig peritoneal exudate cells (PEC) in the presence of the antigen, and migration of PEC was measured after incubation at 37°C for 48 hours. The MIF response was variable throughout 6-77 days after infection using a mean of 58 infective larvae of Taiwanese strain. Seven infected rats on days 15, 20, 48, and 49 were positive, while 6 other infected rats were negative on days 6, 34, 35, 37, 63 and 77. Furthermore, the MIF results did not necessarily agree with dermal reactivity. The factor(s) responsible for this inconsistency were not known. In the indirect test, the supernatant from 48-hour cultures of lymphocytes grown in the presence of the antigen was tested against migration of normal guinea pig PEC. When lymphocytes from cervical lymph nodes were used, 3 out of 8 rats tested during 7-37 days postinfection were positive, whereas with cells from mediastinal nodes of 5 out of 7 rats (71%) examined at 29-51 days of infection yielded positive MIF test.

A slightly different method of direct testing used by Damrong-At (1977) in which migration of PEC from rats infected with infective larvae of the Thailand strain in the presence of either somatic antigen or female ES antigen was used. The results are presented in Tables 2 and 3. Although variation was seen in each study group, the MIF test was positive 6-14 weeks after infection. The positive MIF test was more pronounced when female ES antigen was used. Findings from these 2 groups of investigators could not be justifiably compared, owing to difference in the techniques used, the difference in the strain of the parasite, the difference in the source of PEC used in the test, and the difference in the concentration of the antigen used. Nevertheless, it is clear that cell-mediated immune response develops in rats with angiostrongyliasis, but the role of CMIR in protective immunity against angiostrongyliasis in the rat requires further study.

Table 2

Summary of the effect of female adult worm antigen on the migration of peritoneal exudate cells from rats infected with *Angiostrongylus cantonensis**

Duration of infection	Number of rats	Mean+	S.E.	P – value (normal VS infected)
Uninfected control	5	97.6	4.8	
4 – 5 wks	7	85.0	4.0	<.05
6 wks	6	79.6	4.3	<.025
10 – 13 wks	6	88.7	5.5	>.05
24 – 28 wks	6	92.1	3.6	>.05

*Taken from Damrong –At, A. (1977) M.Sc. Thesis, Mahidol University.

+Mean value of cell migration in the presence of antigen (The coresponding value of cell migration in the absence of antigen was taken as 100).

Guinea Pigs. *The MIF response was consistently positive 12-35 days postinfection in guinea* pigs infected orally with a mean of 70 third-stage larvae of the Taiwan strain of *A. cantonensis* (Yoshimura *et al.*, 1976). In contrast to the study on blast transformation of lymphocyte in rats, lymphoid cells from cervical lymph nodes, as well as, those from the spleen from infected guinea pigs produced MIF. In addition, the results of MIF response correlated well with delayed skin test activity, but infected guinea pigs failed to produce antibody measured by the indirect haemagglutination and the gel diffusion tests.

Table 3

Summary of the effect of female ES antigen on the migration of peritoneal exudate cells from rats infected with *Angiostrongylus cantonensis**

Duration of infection	Number of rat	Mean+	S.E.	P. value (normal VS infected)
Uninfected control	7	92.6	4.0	
4 – 5 wks	6	88.8	7.3	>0.5
8 wks	6	75.4	2.1	<0.005
12 – 14 wks	8	67.4	8.9	<0.025
20 wks	4	77.2	6.1	<0.05

*Taken from Damrong –At, A (1977) M.Sc. Thesis, Mahidol University.

+Mean value of cell migration in the presence of antigen (the corresponding value for cell migration in the absence of antigen was taken as 100).

IMMUNODIAGNOSIS OF HUMAN ANGIOSTRONGYLIASIS

Complement fixation (CF) test. The CF test for angiostrongyliasis was first reported by Anderson *et al.* (1962) using somatic and metabolic antigens of *A. cantonensis.* With somatic antigens, positive results were observed in sera from individuals with eosinophilic meningitis, among Tahitians with no history of the disease and among children from non-endemic tropical areas. The test was also performed with cerebrospinal fluid (CSF). CSF from 9 individuals diagnosed as having eosinophilic meningitis were tested with metabolic antigen and 8 gave a reaction and one showed a weak reaction. Fewer reactions were obtained with somatic antigens. Despite some positive finding either with metabolic or somatic antigens, the authors felt the results to be inconclusive. Mishra and Benex (1970) were dissatisfied with the CF test against immune rat sera. Three extracts of worms with protein concentrations of 3, 4 and 7 mg per ml were shown to be anti-complementary even when diluted 1/20.

Indirect Haemagglutination (IHA) test with somatic antigen. The IHA test has been used by several investigator for the diagnosis of human angiostrongyliasis and some of the results are summarised in Table 4. While Kamiya *et al.* (1973); Kamiya (1975) recorded a high percentage of seropositivity (93%) in Thai patients with eosinophilic meningitis, the seropositivity in the series of patients studied by Tungkanak was only 24%. The reason for this discrepancy was unknown. Subsequent work by Taiwanese and Japanese investigators using antigen purified by DEAE-cellulose chromatography lend support to the contention that this test has some value for the diagnosis of angiostrongyliasis. In 4 patients with the history of eating raw *Achatina falica,* the IHA serum titer was 1:128 in 2, and 1:256 in 2 others (Chen, 1975). Sato *et al.* (1975) showed that sera from 2 patients on Okinawa who had a histories of eating livers from toads developed IHA titers of 1:4096 and 1:256, and 6 months later the titers decreased to 1:1024 and 1:128, respectively. In 2 other patients with histories of eating raw slugs 10 and 53 months earlier, titers of 1:64 and 1:16, respectively, were obtained.

Table 4

Indirect haemagglutination tests in angiostrongyliasis

No.	Source and dilution of antigen for sensitization of sheep red blood cells	Significant titer	Patients diagnosed clinically as eosinophilic meningitis		Control		Ref.
			Serum	CSF			
			No. positive/ No. examined (%)	No. positive/ No. examined (%)	Serum	CSF	
1.	1:16,000 dilution of dry weight of adult female worm	1:32 for serum 1:4 for CSF	14/15 (93)	7/8 (88)	15/159 (9.4)	1/53 (2)	Kamiya (1973) (1975)
2.	Some as above, but the Ag used was 4 times more diluted	at least 4 fold increase from that of non-sensitised cells	4/17 (24)	5/17 (29)	ND*	ND	Tungkanak *et al.*, (1972)
3.	Extract of adult worms purified by DEAE cellulose chromatography	1:40	4/4 (100)	ND	ND	ND	Chen (1975)

* ND = Not done

The IHA test has been used in a seroepidemiological study among patients with suspected Japanese encephalitis on Taiwan (Suzuki *et al.*, 1973). The antigen used was the crude extract of adult worms and a titer of 1:40 or more was considered positive. A seropositivity rate of 6% among 800 cases examined was found. However, false positive reaction owing to possible cross-reactions with other helminths had not been ruled out.

Such false positive reactions have been documented in 9.4% of healthy Thai blood donors (Kamiya *et al.*, 1975). This is likely to be due to cross-reaction with other parasitic infections particularly nematodes. Such cross-reactivity had been reported by Suzuki *et al.* (1975) in sera of rats infected with *Ascaris suum* and *Toxocara canis*, but little or no corss-reactivity was found with *Anisakis* sp., *Dirofilaria immitis*, and *Paragonimus westermani*. For a specific IHA test, it is desirable to purify the antigen to eliminate cross-reacting antigens. With gel filtration through Sephadex G-200, Kamiya *et al.* (1973) showed that most of the antigenicity for the IHA test was associated with the first elution peak. With DEAE-cellulose chromatography, the fraction with good IHA activity was recovered from the peak eluted with phosphatebuffer containing 0.1 M NaCl (Sato *et al.*, 1974). Chen (1975) purified the IHA antigen from a crude extract of adult worms by DEAE-cellulose chromatography and the associated antigens derived from the rat were removed by affinity chromatography (i.e. sepharose coupled with rabbit anti-rat serum). A more extensive purification proceduré was reported by Suzuki *et al.* (1975) using various steps of affinity chromatography although purified antigen showed good promise in the IHA test for human angiostrongyliasis, the application of such antigen has not been documented.

The use of ES antigen in the IHA test for angiostrongyliasis has been reported only in experimental rats showing that only female ES, and not male ES antigen, was active (Kamiya, 1975). Positive reaction was evident in sera from infected rats only 6 or more weeks after infection thus supporting the contention by Jacobs and Lunde (1965) that serum IHA activity was associated with fecundity.

Gel diffusion test and immunoelectrophoresis (IEP). Bouthemy *et al.* (1972) reported that 13 out of 16 serum samples from 8 patients with eosinophilic meningitis were positive in the IEP test against the extract of adult worm with the number of bands ranging from 1-4. (4 bands 1; 3 bands 3; 2 bands 3; 1 band 6). Some of these sera were also reactive against antigens from other helminths (4 against *D. viteae*, 2 against *T. canis* and 1 against *A. suum).* Kamiya (1975) found that 12 out of 15 patients (80%) with eosinophilic meningitis were positive in the IEP test using the extract of adult female worms, whereas sera from 100 blood donors and 6 patients with opisthorchiasis were negative. Tharavanij *et al.* (1975, unpublished observation) found that 13/20 (72%) patients with eosinophilic meningitis were positive in the IEP test against the extract of adult female worm, with the numbers of bands ranging from 1-4 (4 bands 1; 3 bands 1; 2 bands 5 and 1 band 6). Furthermore 3 of 20 sera (15%) from patients with gnathostomiasis were positive with only one band. Sato *et al.* (1974) found that sera from 2 patients tested within a month after eating toad livers were positive producing 6 and 2 precipitating bands in the gel diffusion test. Sera from 2 other patients tested at 10 and 53 months after eating raw slug were negative.

Enzyme-linked immunosorbent assay (ELISA). The test originally developed by Engvall and Perlmann (1972) was employed for the diagnosis of angiostrongyliasis by Cross (1978) using the antigen prepared from the larvae recovered from rat brains. All sera (some of them were paired) from 6 patients with angiostrongylid induced eosinophilic meningitis

(3 parasitologically confirmed) were positive with ELISA values ranging from 13-71. Sera from 3 Americans with histories of eating raw snails on Okinawa and with clinical eosinophilic meningitis were also positive with an ELISA values of 6.6, 13, and 33. In the healthy controls and in patients with other parasitic infections e.g. amoebiasis, intestinal capillariasis, bancroftian filariasis, and schistosomiasis the ELISA value was very low ranging from 0-6.3, average 2.6. Although the ELISA method showed great promise, the author suggested additional testing and refinement of the test be done before adoption for routine use.

Detection of the antigen in the CSF. Theoretically, detection of the antigen would be more related to clinical illness than detection of serum antibody. Unfortunately, this approach has not been attempted in human angiostrongiliasis. The possible usefulness of this approach was exemplified in monkeys experimentally infected with *Angiostrongylus* larvae (Chen *et al.*, 1973). The antigen in the CSF was detected by indirect haemagglutination test in which tanned sheep red blood cells were sensitized with rat IgG antibody instead of *A. cantonensis* antigen. The antigen titer reached the highest titer at 10 days after infection and declined gradually. After 20 days the antigen was no longer detectable.

Intradermal test. Immediate type of skin reaction had been reported, for the most part for angiostrongyliasis. Alicata and Brown (1962) performed skin test by injecting 1:10,000 dilution of dried adult worm extract in physiological saline in 0.05 ml. Positive reactions were found in Tahitian patients with eosinophilic meningitis and residents in Tahiti, whereas controls were negative. Subsequently, Alicata and Jindrak (1970) reported that in areas with increased parasitism, tests using the crude antigen were not reliable. Kagan and Zaiman (1964) prepared an extract of lyophilised adult worm in modified Coca's solution with a protein-nitrogen concentration of 8 ug/ml. A volume of 0.05 ml was injected intradermally and the result read after 15 minutes. The results were considered positive with a wheal diameter of 0.2 cm^2 or greater in 16 out of 88 (18%) Puerto Rican patients with tuberculosis in New York City but none of the 259 non-Puerto Ricans were positive. They concluded that the positive reactions among Puerto Ricans were probably due to non-specific cross-reactions with other helminthic infections, since *A. cantonensis* has not been found in Puerto Rico. Consequently in 2 subsequent reports on the intradermal test the investigators employed purified antigen. Chen *et al.* (1974) used antigen purified by DEAE-cellulose chromatography with the protein nitrogen content of 20 µg per ml and a volume of 0.02 ml was inoculated intradermally. The wheal diameter of 9 mm or larger measured at 15 minutes was considered as a positive reaction and positive reactions were obtained in 289 out of 1459 Taiwanese residents (20%). The positive reaction could not be due wholly to angiostrongyliasis, since the skin test with a highly purified antigen was positive in 1.9% of residents in Niigata Prefecture, Japan where *A. cantonensis* had never been observed (Suzuki *et al.*, 1975).

SUMMARY

Specific acquired immunity to angiostrongylus infection has been demonstrated in animals infected with sublethal dose of infective larvae and in animals immunised with irradiated larvae or secretary-excretory antigen of adult female worms. A substantial number

of worms in immune animals are dead and those that survive have stunted growth. The mechanism whereby immunity is operated is not clearly understood, but humoral and cellular factors may have important roles. Humoral antibody causes microprecipitation around orifices of the worms and interferes with their metabolism. The antibody response can be detected as early as 5 days after infection and remains positive for a considerable period of time. The haemagglutinating antibody appears to be associated with maturity and fecundity of adult female worms. Cell mediated immune responses have been demonstrated in experimentally infected rats or guinea pigs but the results so far reported showed some variation with respect to the time course of infection, the source of lymphocytes used in the test, and the species of animals used in the study.

Several serological diagnostic tests for human angiostrongyliasis have been reported, in which somatic antigens from adult worms are mostly used. Some of these tests show good promise but more extensive studies and evaluations are needed. It appears however that the ELISA test using the antigen prepared from the larvae recovered from the brain is most promising; the test employing purified antigen from the adult worms should also be of value. In addition, detection of the antigen especially the ES antigen in the CSF should be attempted, the success of which would be of benefit in the diagnosis of human infection.

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