STUDIES OF *ANGIOSTRONGYLUS MALAYSIENSIS* (NEMATODA, METASTRONGYLIDAE) IN PENINSULAR MALAYSIA: NATURAL INFECTION IN FRESHWATER SNAILS AND RODENTS IN RICEFIELDS AND INFECTIVITY EXPERIMENTS

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INTRODUCTION

The principal intermediate hosts of *Angiostrongylus malaysiensis* are land snails and slugs (Lim and Heyneman, 1965; Lim *et al.*, 1965; Bisseru and Verghese, 1970). Freshwater snails have also been found naturally infected with the parasite but the infection rates and worm-loads were low as compared to the land molluscs (Lim and Krishnansamy, 1970). In Thailand freshwater snails, particularly *Pila* spp., were found to be one of the important intermediate hosts of *A. cantonensis* as a cause of human eosinophilic meningoencephalitis in man (Harinasuta *et al.*, 1964). 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). The present study deals with two species of edible freshwater snails, *Pila scutata* and *Bellamyia ingallsiana*, common in ricefields in Peninsular Malaysia. Attempts were made to determine whether these snails serve as intermediate hosts of the parasite. Furthermore, rats trapped in ricefields, where these snails were collected, were examined for *A. malaysiensis*. In addition, experimental infectivity studies were done on clean snails collected from the field by exposing to infected rat faeces and by feeding with live slugs, *Microparmarion malayanus* presumably harbouring the parasite in nature.

MATERIALS AND METHODS

Study areas: Five study areas where rice was grown were selected from the states of Kelantan, Trengganu, Kedah, Selangor and Malacca, viz., Kampung Machang (6° 51′ N, 102° 8′ E), Kampung Manir (51° 9′ N, 103° 6′ E), Kuala Jerlun (6° 21′ N, 100° 21′ E), Kampung Beranang (3° 10′ N, 102° 5′ E), and Kampung Alai (2° 15′ N, 102° 25′ E)(see Map, Fig. 1).

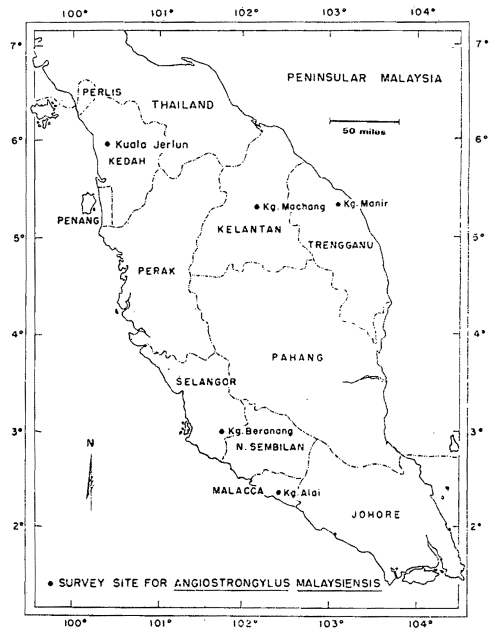

Fig. 1—Map showing survey sites for *Angiostrongylus malaysiensis* in Peninsular Malaysia.

Field studies: Collecting of *Pila* and *Bellamyia* snails and trapping of rats were done for seven days in each study site. A total of 500 snails of each species were collected and examined from each area. Most of the snails

were brought back to the laboratory in Kuala Lumpur for processing, but examination of the rats was carried out in the field. The rats were killed with chloroform and dissected for presence of the worms in the brains, spinal cords, hearts and lungs. The worms recovered were fixed and preserved in 70% alcohol and later transferred to 5% glycerol in 70% alcohol, and cleared in lactophenol for examination. In the laboratory, the snails were individually examined; the hard shell was removed and the body was minced into fine pieces, with a pair of fine surgical scissors, in physiological saline and then digested in artificial gastric juice for four hours at 37°C before examination for *A. malaysiensis* larvae.

Experimental studies: 350 *Pila scutata* and 250 *Bellamyia ingallsiana* snails were collected from a mining pool in Cheras, Kuala Lumpur. 100 snails of each species were dissected and were found to be free of *A. malaysiensis.*

The remaining 250 *P. scutata* and 150 *B. ingallsiana* were divided into batches of 50 snails each for the following experiments. The first experiment was on 50 snails of each species fed with 25 grams of infected faeces obtained from experimentally infected inbred albino Norway rats with *A. malaysiensis.* Two batches each of 50 *P. scutata* and 50 *B. ingallsiana* were placed in separate aquaria, and each of these batches of snails were fed with infected faeces continuously for 6 days. Thereafter, the snails were fed with clean lettuce leaves. The first batch each of experimentally infected 50 *P. scutata* and 50 *B. ingallsiana* were killed at 15 days from the initial day of exposure to the infected faeces. The second batch of snails were killed at 20 days later. Two batches of 50 snails of each species were used as control. The snails were fed with clean lettuce leaves and were killed 20 days later.

The second experiment on 50 *P. scutata* snails were fed with live slugs, *Microparmarion malayanus* presumably harbouring the parasite in nature. These slugs were collected from an oil-palm estate where natural infection in these slugs was about 45% (Lim and Heyneman, 1965). 15 live slugs were fed to each of these snails daily for 6 days. Subsequently the snails were fed with clean lettuce until they were killed at 25 days from the initial day of exposure to the live slugs. A batch of 50 *P. scutata,* used as control, were fed with clean lettuce leaves only and killed 15 days later.

The experimental snails were individually examined. The hard shell of the snail was removed. The body was minced with a pair of surgical scissors and digested in artificial gastric juice for four hours at 37°C and later examined for *A. malaysiensis* larvae. The larvae collected were fed to albino rats by means of a Pasteur pipette and subsequently to recover adult worms for confirmation of the parasite.

Adjusted Chi-square and Student's 't' tests were used for statistical analysis.

RESULTS

Natural infection in freshwater snails: A total of 5,000 snails, consisting of 2,500 each of *P. scutata* and *B. ingallsiana,* were examined. Table 1 shows the natural infection rates and worm loads in the snails with *A. malaysiensis.* The infection rate in *Pila* snails from Kelantan was found to be significantly higher than in those examined from Trengganu, Selangor or Malacca, but no marked difference was found between Kedah and Kelantan. The rate in Kedah was significantly higher than in those examined from Trengganu, Malacca or Selangor. Infection rate in the snails from Trengganu was significantly higher than in those examined from Selangor but no difference was observed between Trengganu and Malacca, similarly no difference in the infection rate in the snails between

Table 1

Prevalence of *Angiostrongylus malaysiensis* in freshwater snails collected from ricefields in Peninsular Malaysia.

Locality	Species	No. exam.	No. pos.	Infection rate %	Larvae recovered		
					Total	$\bar{x} \pm SD$	Range
Kampung Machang (Kelantan)	*Pila scutata*	500	49	9.8	908	18.53±20.45	3-82
	Bellamyia ingallsiana	500	17	3.4	200	11.76±6.34	2-22
Kampung Manir (Trengganu)	*Pila scutata*	500	22	4.4	469	21.32±13.98	2-55
	Bellamyia ingallsiana	500	6	1.2	67	11.17±7.19	3-22
Kampung Jerlun (Kedah)	*Pila scutata*	500	42	8.4	797	18.98±16.73	2-52
	Bellamyia ingallsiana	500	14	2.8	151	10.78±6.01	3-22
Kampung Beranang (Selangor)	*Pila scutata*	500	11	2.2	238	21.63±15.82	3-46
	Bellamyia ingallsiana	500	3	0.6	59	19.67±15.70	2-32
Kampung Alai (Malacca)	*Pila scutata*	500	12	2.4	280	23.33±25.60	5-101
	Bellamyia ingallsiana	500	4	0.8	61	15.25±9.78	3-25

Malacca and Selangor. There was no statistical difference in the mean worm-load per infected snail between each of these study areas.

The infection in *B. ingallsiana* snails from Kelantan was found to be significantly higher than in those examined from Trengganu, Malacca, Selangor but no difference was observed between Kelantan and Kedah. Similarly, the infection rate in this snail from Kedah was observed to be significantly higher than in those examined from Trengganu, Selangor and Malacca, but no difference between Selangor and Malacca. The mean worm-load in infected snails in the states of Selangor and Malacca was slightly higher than in those examined in the states of Kelantan, Trengganu and Kedah, but there was no statistical significant difference.

In order to evaluate which species of snails is a more important intermediate host for the parasite, an overall calculation was made for each species. The overall infection rate in *P. scutata* was 5.4% with a mean worm-load per infected snail of 19.7 ± 18.4 as compared with 1.8% and 12.2 ± 7.5 in *B. ingallsiana* respectively. The infection rate in *P. scutata* was not only found to be significantly higher than in *B. ingallsiana* but also the mean worm load.

Natural infection in wild rats: A total of 932 wild rats of four species, *Rattus tiomanicus (= jalorensis)*, *R. argentiventer*, *R. exulans* and *R.r. diardii* were trapped in the five study areas. These consisted of 151 rats from Kelantan, 204 from Trengganu, 242 from Kedah, 160 from Selangor and 175 from Malacca. Of these 34.9% (326) were found naturally infected with *A. malaysiensis*.

Table 2 shows the prevalence of natural infection in these wild rats with the parasite in each of the study areas. The overall infection rate in the four species of rats from Kelantan was 34.43%, Trengganu 36.27%; Kedah 37.60%, Selangor 26.80%, and Malacca 33.71%. The infection rates in these rats were statistically found to be not significant from one study area to another. The overall mean worm-load per infected rat in Kelantan was 5.0 ± 3.21, Trengganu 5.18 ± 3.02, Kedah 4.50 ± 3.51, Selangor 5.70 ± 3.58 and Malacca 5.22 ± 3.65, and there was no statistical significant difference between them from each study area.

Table 2

Prevalence of *Angiostrongylus malaysiensis* in rats collected from ricefields in Peninsular Malaysia.

Locality	Species	No. exam.	No. infected	Infection rate %	Worms recovered		
					Total	Mean no. of worms per rat	Range
Kampung Machang (Kelantan)	*Rattus tiomanicus (= jalorensis)*	30	12	40.0	81	6.75±4.66	2-18
	Rattus argentiventer	77	28	36.3	120	4.29±2.52	1-11
	Rattus rattus diardii	29	8	27.6	48	6.0 ±2.14	3-9
	Rattus exulans	15	4	26.7	11	2.75±0.96	2-4
Kampung Manir (Trengganu)	*Rattus tiomanicus*	42	15	35.7	100	6.67±4.12	2-16
	Rattus argentiventer	85	33	38.8	170	5.16±2.88	1-11
	Rattus rattus diardii	39	14	35.9	76	5.43±1.99	2-8
	Rattus exulans	38	12	31.6	49	3.09±1.37	1-5
Kampung Jerlun (Kedah)	*Rattus tiomanicus*	37	15	40.5	119	7.93±5.95	2-24
	Rattus argentiventer	107	51	47.6	186	3.65±2.11	1-8
	Rattus rattus diardii	49	11	22.4	59	5.37±3.0	2-11
	Rattus exulans	49	14	28.6	45	3.22±2.04	1-8
Kampung Beranang (Selangor)	*Rattus tiomanicus*	62	17	27.4	130	7.65±4.17	2-18
	Rattus argentiventer	32	12	37.5	53	4.42±2.19	1-8
	Rattus rattus diardii	35	8	22.9	44	5.50±3.21	2-12
	Rattus exulans	31	6	19.4	18	3.0 ±1.27	2-5
Kampung Alai (Malacca)	*Rattus tiomanicus*	56	18	32.1	141	7.84±4.82	2-21
	Rattus argentiventer	33	17	51.5	55	3.24±1.92	1-8
	Rattus rattus diardii	47	15	31.9	79	5.27±2.60	2-11
	Rattus exulans	39	9	23.1	33	3.67±1.22	2-6

Since there was no statistically significant difference in the overall infection rate and mean worm-load in rats when compared with each of the five study areas, therefore the parasitological data from all infected rats in the study areas were combined and calculated according to specific groups. The infection rate in *R. tiomanicus* was 33.92%, *R. argentiventer* 42.22%, *R.r. diardii* 28.14%, and *R. exulans* 25.71%. Other than the rate in *R. argentiventer* was found to be significantly higher than in *R.r. diardii* and *R. exulans*, no statistical difference was observed in *R. tiomanicus* with *R. argentiventer* or *R.r. diardii* or *R. exulans*. Similarly, no significant difference in the rate was found between *R.r. diardii* and *R. exulans*. The mean worm load per infected *R. tiomanicus* was 6.75 ± 4.66, *R. argentiventer* 4.29 ± 2.52, *R.r. diardii* 6.00 ± 2.14, and *R. exulans* 2.75 ± 0.96. The mean worm-load in *R. tiomanicus* was found to be significantly higher than in *R. argentiventer* and *R. exulans*, but no marked difference between *R. tiomanicus* and *R.r. diardii*. The mean worm-load in *R.r. diardii* was significantly higher than in *R. exulans*, but no difference between *R.r. diardii* and *R. argentiventer*. Similarly, the mean worm-load in *R. argentiventer* was also found to be significantly higher than in *R. exulans*.

Infectivity studies by exposure to infected rat faeces: 20% (10/50) of *P. scutata* and 10% (5/50) of *B. ingallsiana* became infected 15 days after the initial exposure to infected rat faeces (Table 3). The infection rate in *P.*

Table 3

Infectivity study of *Pila scutata* and *Bellamyia ingallsiana* exposed to faeces from experimentally infected albino rats with *Angiostrongylus malaysiensis*.

Snails	No. of snails used	No. of days faeces fed to snails	No. of days snails killed after feeding	No. of snails infected	Larvae recovered			Per cent infected
					Total	Mean $\bar{x} \pm SD$	Range	
P. scutata	50	6	15	10	228	22.80 ± 14.70	8-48	20
B. ingallsiana	50	6	15	5	48	9.60 ± 7.30	3-18	10
P. scutata	50	6	20	11	189	17.18 ± 10.75	7-26	22
B. ingallsiana	50	6	20	4	39	9.75 ± 6.80	3-17	8
CONTROL								
P. scutata	50	-	20	0	-	-	-	-
B. ingallsiana	50	-	20	0	-	-	-	-

scutata was found to be significantly higher than *B. ingallsiana*. The control batch of *P. scutata* killed 20 days later were found clean without *A. malaysiensis* larvae (Table 3).

228 ($\bar{x} \pm SD = 22.80 \pm 14.70$) and 48 (9.60 ± 7.30) larvae were recovered from 10 infected *P. scutata* and 5 *B. ingallsiana* respectively (Table 3). The mean worm-load per infected snail of *P. scutata* was found to be significantly higher than that of *B. ingallsiana*. The larvae recovered from *P. scutata* were fed to two albino rats, and those from *B. ingallsiana* to one albino rat. These experimental rats were killed at 30 days and none were found with adult worms. Hence, the larvae failed to develop in these rats.

Experimental snails that were killed 20 days after infection of which 22% (11/50) of *P. scutata* and 8% (4/50) of *B. ingallsiana* were infected (Table 3). Again *P. scutata* was observed to have a significantly higher rate than *B. ingallsiana*. The control batch of *B. ingallsiana* killed 20 days later were all free of *A. malaysiensis* larvae (Table 3).

A total of 189 ($\bar{x} \pm SD = 17.18 \pm 10.75$) and 39 (9.75 ± 6.80) larvae were recovered from 11 infected *P. scutata* and 4 *B. ingallsiana* respectively. The mean worm-load in *P. scutata* was significantly higher than in *B. ingallsiana* ($t = 2.15$, $0.05 > p > 0.02$). The recovered larvae from *P. scutata* and from *B. ingallsiana* were fed to one albino rat each. The experimental rats were killed 30 days after infection. 33 adult worms were recovered from the lungs and heart of the rat infected with larvae from *P. scutata*, and 8 adult worms from the lungs of the rat infected with larvae from *B. ingallsiana*.

Infectivity studies by feeding on live slugs: In the laboratory *P. scutata* snails were observed to feed on slugs which were accidentally dropped into the aquaria. In the field, *P. scutata* has been observed by one of us also to feed on freshwater crustaceans, particularly shrimps, *Macrobrachium* spp. and small fishes such as *Betta* spp., *Rasbora* spp., and Gouramies. Based on these observations, it was concluded that *Pila* snails are omnivorous than herbivorous in habits, we assumed that this snail could probably obtain its infection in nature by direct ingestion of slugs which may harbour *Angiostrongylus*.

To confirm the aforementioned observations, experiments were carried out by feeding presumably clean *P. scutata* snails with presumably infected slugs collected directly from

Table 4

Infectivity study of *Pila scutata* and *Bellamyia ingallsiana* fed on *Microparmarion malayanus* presumably with *Angiostrongylus malaysiensis*.

Snails	No. of snails used	No. of live slugs fed to snails	No. of days slugs given	No. of days snails killed after feeding	No. of snails infected	Larvae recovered			Per cent infected
						Total	Mean $\bar{x} \pm SD$	Range	
P. scutata	50	15	6	25	20	733	36.65±29.75	9-94	40
CONTROL									
P. scutata	50	-	-	25	0	-	-	-	-

the field. The results show that 30% (15/50) of the snails were infected with the parasite 25 days after feeding with slugs. A total of 733 *Angiostrongylus* larvae were recovered from these infected snails with a mean worm-load of 36.65 ± 29.75 per infected snail (Table 4).

The larvae were fed to three albino rats with 250, 250 and 233 larvae each. These experimental rats were killed 30 days after infection, and 61, 76 and 48 adult worms, confirmed as *A. malaysiensis*, were recovered from each of these rats. All 50 *P. scutata*, used as control, were dissected 25 days later and were found free of *Angiostrongylus*.

DISCUSSION

The natural rodent hosts of *A. malaysiensis* are field and house rats in Peninsular Malaysia (Lim and Heyneman, 1965), and also a few species of forest rats (Lim, 1972), but the field rats appear to be better hosts than the forest rats (Lim *et al.*, 1965). Apart from the ricefield rat, *R. argentiventer*, there are three other species of field and house rats, *R. tiomanicus*, *R. exulans* and *R.r. diardii* which are intermittent residents of ricefield habitat (Lim, 1973). In the present investigation all these four species were trapped and found with natural infection of *A. malaysiensis*. Statistical analyses of the overall infection and mean worm-load of the four species of rats in the different study areas were found to be insignificant.

However, infection rate in individual species of rats revealed that *R. argentiventer* and *R. tiomanicus* have a higher rate than *R.r. diardii* and least of all *R. exulans*. The higher rate in *R. argentiventer* and *R. tiomanicus* could be due to habitat preference of the host. These two species are primarily field rats and confined to the open only, thus their chances of contact with the intermediate molluscan hosts of the parasite are greater. In the case of *R.r. diardii*, primarily a house rat, which is an intermittent resident in the open and the fact that 28% of the specimens examined were found harbouring the parasite indicates that snails are probably an important source of its diet. *R. exulans* on the other hand, is not only a house rat but also extends its range from field to forest. The low rate in this species could be due to sampling being limited to ricefield habitat only in the present survey. The varying degree in the mean worm-load in all these four species of rats is not due to the susceptibility status of the rodent hosts but more to the degree of infectivity and density of the parasite in the molluscan intermediate hosts consumed.

The primary intermediate hosts of *A. malaysiensis* in ricefields are *P. scutata* and *B. ingallsiana*. The degree of infection rates in *P.*

scutata appears to vary in different localities sampled with higher rates in the north (Kedah) and east (Kelantan and Trengganu) and lower rates in the central (Selangor) and south (Malacca) of Peninsular Malaysia. The higher infection rate in these snails in the northern and eastern parts of the country could be due to double-cropping of padi in both these regions and therefore provide stable habitats for the maintenance of the freshwater snails throughout the year. On the other hand, the ricefields sampled in the central and southern regions have single-cropping only. Thus, the maintenance of freshwater snails inhabiting these areas was disrupted when the fields after harvesting were dried up for several months a year.

Overall comparative studies of the two species of freshwater snails in these areas revealed that *P. scutata* had a higher infection rate as well as mean worm-load than *B. ingallsiana*. The result indicates that *P. scutata* is probably a better receptive host of the parasite than *B. ingallsiana*. The findings of *P. scutata* being a better intermediate host of *A. malaysiensis* support the preliminary observation of this snail by Lim and Krishnansamy (1970).

Laboratory infections in both species of *P. scutata* and *B. ingallsiana* infected with rat faeces, revealed that *P. scutata* had a significantly higher rate of infection and greater mean worm-load than that of *B. ingallsiana*. These experiments further support our field findings of *P. scutata* being a better intermediate host of *A. malaysiensis*. Observations in the field revealed *P. scutata* to be carnivorous in feeding habits. This is reinforced by the present experimental results that the snail can be infected by feeding it with infected slugs, thus suggesting a second source of infection which may occur also in nature. It appears that *P. scutata* is not only capable in acting as a direct intermediate host by ingesting first-stage larvae in faeces of infected rats, but it also can act as paratenic host of the parasite similar to the crustacean, *Macrobrachium* sp. and freshwater and marine fish (Alicata and Brown, 1962; Wallace and Rosen, 1967).

The first-stage larvae recovered from *P. scutata* and *B. ingallsiana*, 15 days after exposure to infected rat faeces, failed to develop in experimental albino rats. On the other hand, larvae recovered from these two species of snails, 20 days later, succeeded in infecting the rats. Wallace and Rosen (1969) demonstrated larvae of *A. cantonensis* developed in *Biomphalaria glabrata* to infective stage at 18 days after infection. These differences in the developing stages of the larvae between *A. malaysiensis* and *A. cantonensis* can be due to (1) that the two worms being different species probably reflect the pathogenicity difference of each species; (2) the different species of experimental molluscan hosts could have affected the rate of development in these two worms, and (3) that comparison in the rate of development between these two worms is inadequate particularly so in *A. malaysiensis* because none of the experimental *Pila* snails were dissected 18 days after exposure to the first-stage larvae. Further studies are being carried out by using *Pila* snails under laboratory conditions in elucidating the life-cycle of *A. malaysiensis*.

SUMMARY

A survey for *Angiostrongylus malaysiensis* was carried out on the definitive rodent and intermediate freshwater molluscan hosts in ricefields of five states of Peninsular Malaysia. The primary intermediate hosts of *A. malaysiensis* in ricefields are *Pila scutata* and *Bellamyia ingallsiana*. The degree of infection rates in these snails vary in different localities sampled with higher rates in the north (Kedah) and east (Kelantan and Trengganu) and lower rates in the central (Selangor) and south

(Malacca) of Peninsular Malaysia. Overall comparative studies of these two species of freshwater snails in these areas revealed that *P. scutata* had a higher infection rate as well as mean worm-load than *B. ingallsiana.*

Four species of definitive hosts, *Rattus tiomanicus*, *R. argentiventer*, *R. exulans* and *R.r. diardii* were found naturally infected with *A. malaysiensis.* There was no significant difference in the infection rates as well as the mean worm-load in these rats between each of the areas sampled. However, infection rate in individual species of rats revealed that *R. argentiventer* and *R. tiomanicus* had a higher rate than *R.r. diardii* and least of all *R. exulans.*

Among the two species of experimental freshwater snails, *Pila scutata* and *Bellamyia ingalisiana*, the former species was found to be a better experimental molluscan host for *A. malaysiensis* than the latter not only in the infection rate but also in the recovery of mean worm-load. 20-day old larvae recovered from experimental molluscs were found to be infective to inbred albino Norway rats whereas 15-day old larvae were not infective.

In nature, *P. scutata* was observed to feed on small fishes such as *Betta* spp., *Rasbora* spp., and Gouramies. Experimentally, the snail had shown to feed on live slugs. Larvae recovered from the snails 25 days after feeding on live slugs were infective to albino rat. *P. scutata* has been established to be not only an intermediate host but also could probably act as a paratenic host of *A. malaysiensis.*

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REFERENCES

ALICATA, J.E. and BROWN, R.W., (1962). Observations of the method of human infection with *Angiostrongylus cantonensis* in Tahiti. *Canad. J. Zool., 40* : 755.

BISSERU, B. and VERGHESE, T., (1970). Natural infection of the land mollusc *Achatina fulica* with *Angiostrongylus cantonensis* in Malaya. *Southeast Asian J. Trop. Med. Pub. Hlth., 1* : 294.

CHANG, P.-K., CROSS, J.H. and CHEN, S.S.S., (1968). Aquatic snails as intermediate hosts for *Angiostrongylus cantonensis* on Taiwan. *J. Parasit., 54* : 182.

HARINASUTA, C., SETASUBAN, P. and RADOMYOS, P., (1964). Observations on *Angiostrongylus cantonensis* in rats and molluscs in Thailand. *Proc. 1st Med. Conf. Parasit. Dis.*, Bangkok.

LIM, B.L., (1970). Further studies of *Angiostrongylus cantonensis* in Kuching, Sarawak with special reference to molluscan intermediate hosts. *Southeast Asian J. Trop. Med. Pub. Hlth., 1* : 112.

LIM, B.L., (1974). Small mammals associated with rice-fields. *MARDI Res. Bull., 1* : 25.

LIM, B.L., (1974). New host of *Angiostrongylus malaysiensis* Bhaibulaya and Cross, 1971 in Malaysia. *Southeast Asian J. Trop. Med. Pub. Hlth., 5* : 379.

LIM, B.L. and HEYNEMAN, D., (1965). Host-parasite studies of *Angiostrongylus cantonensis* (Nematoda: Metastrongylidae) in Malaysian rodents: natural infection. of rodents and molluscs in urban and rural areas of central Malaya. *Ann. Trop. Med. Parasit., 59* : 425.

LIM, B.L. and KRISHNANSAMY, M., (1970). Preliminary survey for *Angiostrongylus cantonensis* in edible freshwater snails in West Malaysia. *Southeast Asian J. Trop. Med. Pub. Hlth., 1* : 295.

LIM, B.L., OW-YANG, C.K. and LIE, K.J., (1965). Natural infection of *Angiostrongylus cantonensis* in Malaysian rodents and intermediate hosts, and preliminary observations on acquired resistance. *Amer. J. Trop. Med. Hyg., 14* : 610.

WALLACE, G.D. and ROSEN, L., (1967). Studies on eosinophilic meningitis: Experimental infection of freshwater and marine fish with *Angiostrongylus cantonensis. Amer. J. Epidem., 85* : 395.

WALLACE, G.D. and ROSEN, L., (1969). Studies on eosinophilic meningitis: Experimental infection of rats and other homeothermic vertebrates with *Angiostrongylus cantonensis. Amer. J. Epidem., 89* : 331.