NEW HOSTS OF *ANGIOSTRONGYLUS MALAYSIENSIS* BHAIBULAYA AND CROSS, 1971 IN MALAYSIA

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INTRODUCTION

Lim *et al.*, (1965) found slight differences in the morphology of the Malaysian strain of *Angiostrongylus cantonensis* with that of the original description by Chen (1935) and also by Mackerras and Sandars (1955). Later Cross and Fresh (1969) demonstrated that the Malaysian strain caused a different pathology in animals compared with the Thai and Hawaiian strains. Subsequently, the Malaysian strain was described as a new species, *A. malaysiensis* Bhaibulaya & Cross, 1971. The common natural hosts of *A. malaysiensis (=A. cantonensis)* in Peninsular Malaysia are the field rats, *Rattus argentiventer*, *R. tiomanicus*, *R. exulans* and the house rats, *R.r. diardii* and *R. norvegicus* (Lim *et al.*, 1965; Lim and Heyneman, 1965). Natural infection with the parasite in forest rats, *R. muelleri* and *R. bowersi* was reported by Lim *et al.*, (1965), and Mulkit Singh and Cheong (1971) reported the presence of this parasite in another species of forest rat, *R. sabanus*. In the present study, *Angiostrongylus* worms were recovered from six species of forest rats and a species of primitive primate. The adult worms from each of these species of hosts were studied, and compared with *A. malaysiensis* from the field rats and *A. cantonensis* from Formosa.

MATERIALS AND METHODS

Trapping of forest small mammals was carried out in three lowland forests of mixed secondary and primary type of habitats (Subang, Ulu Gombak and Sungei Buloh Forest Reserves) between 18 to 24 miles southeast, northeast, and south of Kuala Lumpur, Selangor, Malaysia. Collection of field rats for recovery of adult worms for comparative studies was carried out in an oil palm estate north of Kuala Lumpur. Collection of all these animals was made from 1971 through 1973.

The animals were killed with chloroform. The brain and spinal cord were removed and examined under the dissecting microscope for presence of developing worms. Hearts, lungs and pulmonary arteries were also examined for the presence of worms.

Adult *Angiostrongylus* worms were preserved in 70% glycerine alcohol and examined either in glycerol or lactophenol in temporary mounts. Measurements were made by occular micrometer. Adult worms of *A. cantonensis* were sent by Dr. J.H Cross from Formosa who recovered these worms from field rats in Taipei.

RESULTS

Ecology of the hosts: Seven of the 18 species of forest mammals examined were found naturally infected with 82 *Angiostrongylus* worms. Of these four species, *Rattus muelleri*, *R. bowersi*, *R. surifer* and *R. whiteheadi* are terrestrial. All these species moved in and out from primary to secondary habitats (Lim, 1970). *R. annandalei* and *R. cremoriventer* are semi-arboreal, the former species being more common in secondary than in the primary habitats, and vice versa in the latter species. The primitive primate, *Tupaia glis* is semi-

This work was supported by a research grant No. M-71-02 from SEAMEO-TROPMED Project.

Table 1

Natural prevalence of *Angiostrongylus* worms in forest animals.

Species of hosts	No. examined	No. positive	Per cent positive	No. of worms per positive host	
				Mean	Range
Rattus muelleri	223	5	2.3	2.4	1-3
Rattus bowersi	156	3	1.9	3	2-4
Rattus surifer	126	4	3.2	6	4-11
Rattus annandalei	114	4	3.5	11	3-26
Rattus cremoriventer	110	1	0.9	3	-
Rattus whiteheadi	118	1	0.8	2	-
Tupaia glis	135	1	0.7	2	-
Total	982	19	1.9	-	-

Table 2

Showing mean and range measurements (mm) of adult female *Angiostrongylus* worms recovered from different species of forest animals.

Hosts	No. of worms	Body		Esophagus		Anus	Vulva	Projection at tip of worm
		Length	Width	Length	Width			
Rattus muelleri	4	28-32	0.34-0.35	0.33-0.34	0.04-0.07	0.15-0.19	0.07-0.08	present
Rattus bowersi	5	15-37	0.33-0.55	0.22-0.32	0.05-0.07	0.14-0.22	0.03-0.06	present
Rattus surifer	11	27.3 (20-31)	0.37 (0.31-0.47)	0.35 (0.33-0.41)	0.05 (0.04-0.06)	0.20 (0.16-0.29)	0.06 (0.06-0.07)	present
Rattus annandalei	28	27.6 (26-31)	0.36 (0.30-0.47)	0.30 (0.28-0.34)	0.05 (0.04-0.07)	0.20 (0.15-0.26)	0.06 (0.05-0.08)	present
Rattus cremoriventer	1	32	0.41	0.36	0.05	0.19	0.05	present
Rattus whiteheadi	1	25.8	0.49	0.42	0.07	0.21	0.07	present
Tupaia glis	1	27	0.39	0.35	0.05	0.18	0.05	present

arboreal, and is common and abundant both in the primary and secondary forests (Lim, 1968).

Natural infection rate with *Angiostrongylus* worms: Of the six species of naturally infected forest rats, infection rate was higher in *R. annandalei*, *R. surifer*, *R. muelleri* and *R. bowersi* than in *R. cremoriventer*, *R. whiteheadi* and *T. glis* (Table 1). *R. annandalei* and *R. surifer* yielded the highest worm-load, and was low in the other species, (Table 1).

Comparative measurements of adult *Angiostrongylus* worms from different species of hosts are shown in Table 2.

Adult female worms: A total of 51 worms recovered from seven species of animals were examined and measured. Mean measurements was calculated for samples which have more than 10 worms, and these were found in three species of rodents, *R. bowersi*, *R. annandalei* and *R. surifer* only. There appeared to be no overall marked differences in the various measurements of these worms recovered from the various species of hosts. However, all these worms had rounded tails each bearing a stout projection at the tip (Fig. 1).

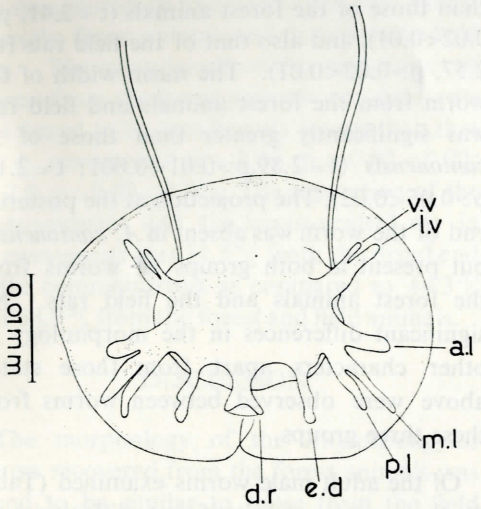

Fig. 1—Showing the separation of the ventro-ventral ray from the latero-ventral ray.

Adult male worms: Measurements of a total of 45 worms recovered from these hosts were made (Table 3). Of these, the length and width of worms recovered from *R. bowersi* were found to be markedly longer and wider than those from other species of hosts. No differences in the measurements were observed in other parts of the worms among these infected hosts. The ventro-ventral ray separates from the latero-ventral ray at between 38.5 to 58.2 per cent of the distance from the common trunk (Fig. 2).

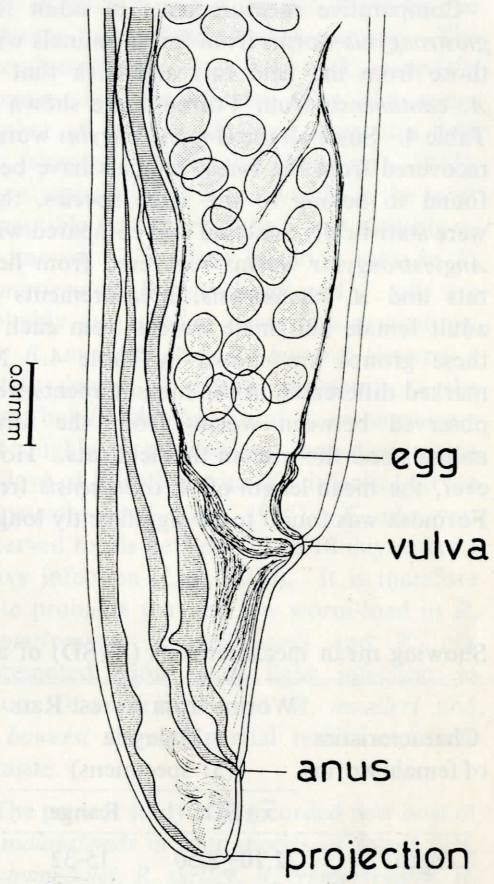

Fig. 2—Showing the projection in the female *Angiostrongylus malaysiensis*.

Table 3

Showing mean and range measurements (mm) of adult male *Angiostrongylus* worms recovered from different species of forest animals.

Hosts	No. of worms	Body		Esophagus		Longest spicule	Distance between v.v/l.v. rays (%)
		Length	Width	Length	Width		
Rattus muelleri	8	18-22	0.23-0.27	0.30-0.39	0.05-0.06	0.85-0.99	37-42
Rattus bowersi	4	19-27	0.33-0.38	0.28-0.34	0.05-0.07	1.07-1.19	35-50
Rattus surifer	13	17.5 (14-22)	0.28 (0.19-0.39)	0.33 (0.30-0.34)	0.04 (0.04-0.05)	0.93 (0.82-1.17)	38.5 (37-40)
Rattus annandalei	16	18.9 (16-22)	0.24 (0.18-0.35)	0.29 (0.24-0.34)	0.05 (0.04-0.06)	0.91 (0.81-0.99)	58.5 (40-75)
Rattus cremoriventer	2	15-22	0.18-0.31	0.28-0.31	0.04-0.06	0.81-1.01	40-52
Rattus whiteheadi	1	19	0.30	0.31	0.05	0.98	52
Tupaia glis	1	18	0.37	0.33	0.05	1.03	48

Comparative measurements of adult *Angiostrongylus* worms from forest animals with those from the field rats and with that of *A. cantonensis* from Formosa are shown in Table 4. Since all the *Angiostrongylus* worms recovered from the forest animals have been found to belong to the same species, they were statistically analysed and compared with *Angiostrongylus* worms recovered from field rats and *A. cantonensis*. Measurements of adult female and male worms from each of these groups are shown in Table 4. No marked differences in the measurements were observed between worms from the forest animals and those from the field rats. However, the mean length of *A. cantonensis* from Formosa was found to be significantly longer than those of the forest animals ($t = 2.41$, $p > 0.02 < 0.01$), and also that of the field rats ($t = 2.57$, $p > 0.02 < 0.01$). The mean width of the worm from the forest animals and field rats was significantly greater than those of *A. cantonensis* ($t = 2.89, p > 0.01 < 0.001$; $t = 2.14$, $p > 0.05 < 0.02$). The projection at the posterior end of the worm was absent in *A. cantonensis*, but present in both groups of worms from the forest animals and the field rats. No significant differences in the morphology of other characters apart from those stated above were observed between worms from these three groups.

Of the adult male worms examined (Table 4) there were no significant differences in

Table 4

Showing mean measurements ($\bar{x} \pm SD$) of adult male and female *Angiostrongylus* worms (mm).

Characteristics of female worms	Worms from Forest Rats Malaysia (51 specimens)		Worms from Field Rats Malaysia (50 specimens)		Worms from Formosa (50 specimens)	
	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range
Length	22.10±8.50	15-32	22.20±2.73	19-29	25.42±3.84	18-34
Width	0.40±0.07	0.30-0.55	0.37±0.05	0.29-0.54	0.34±0.05	0.27-0.40
Es. length	0.32±0.04	0.22-0.41	0.32±0.03	0.28-0.34	0.26±0.04	0.22-0.38
Es. width	0.06±0.01	0.04-0.08	0.04±0.09	0.29-0.58	0.05±0.06	0.04-0.07
Vulva length	0.20±0.01	0.14-0.29	0.18±0.02	0.14-0.21	0.20±0.05	0.15-0.38
Anus length	0.05±0.02	0.03-0.08	0.06±0.02	0.04-0.08	0.05±0.01	0.04-0.08
Projection	Present	-	Present	-	Absent	-
Characteristics of male worms	(45 specimens)		(50 specimens)		(50 specimens)	
	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range
Length	19.68±3.13	14-27	18.26±1.74	17-23	19.54±2.77	17-23
Width	0.27±0.07	0.18-0.39	0.27±0.05	0.19-0.29	0.27±0.04	0.19-0.35
Es. length	0.30±0.04	0.24-0.39	0.31±0.04	0.18-0.34	0.25±0.04	0.22-0.42
Es. width	0.05±0.01	0.04-0.07	0.04±0.01	0.04-0.08	0.05±0.06	0.04-0.06
Spicule length	0.96±0.13	0.81-0.99	0.01±0.06	0.95-1.13	1.04±0.13	0.77-1.27
Separation between v.v/l.v.	44.3%	35-75%	58.9%	35-61.1%	31.53%	16.7-40.7%

the morphological characters between worms from the forest animals and that from the field rats. The mean length of the oesophagus of worms from the forest animals and field rats was found to be significantly longer than that of *A. cantonensis* ($t = 2.45$, $p > 0.05 < 0.02$; $t = 2.0$, $p > 0.05 < 0.02$). The separation of the ventro-venrtal and the latero-ventral ray in *A. cantonensis* was 31.5% from the distal end of the common trunk as compared to 44.3% and 58.9% from the forest and field animals.

DISCUSSION

The morphology of the *Angiostrongylus* worms recovered from the forest animals was found to be similar to those from the field rats, but different from that of *A. cantonensis* from Formosa. The most obvious characters of male *A. malaysiensis* are the shorter spicule length and the position of separation of the ventro-ventral ray from the latero-ventral ray which is at the point of half to less than promixal two thirds of the common trunk (Bhaibulaya and Cross, 1971). In the present study there was no significant difference observed in the spicule length between them, but separation of the rays in *A. cantonensis* was found to be significantly higher (about 1/3 distance from the common trunk). In the female worms, the presence of the projection at the tip of the tail end in *Angiostrongylus* worms from the forest and field animals agrees with the description of *A. malaysiensis* by Bhaibulaya and Cross (1971). Thus, it is concluded that the *Angiostrongylus* worms recovered from the forest animals belong to *A. malaysiensis*.

In comparison with the infection rate of the field rats, *R. argentiventer* (34%) and *R. tiomanicus* (41%) (Lim *et al.*, 1965), natural infection rate in the forest animals (1.9%) was exceptionally low. This was probably attributed to the scarcity of intermediate hosts in forest habitats. The higher rate of natural infection with *A. malaysiensis* in *R. annandalei* and *R. surifer*, could be due to better susceptibility to the parasite. The exceptionally high worm-load in *R. annandalei* (average 11) was greater than the mean number of worms obtained from the field rats (average 8) (Lim and Heyneman, 1965), thus suggesting that *R. annandalei* is the most susceptible host among the forest animals so far known. The lower infection rates in *R. cremoriventer*, *R. whiteheadi* and *T. glis* are probably due to their being less susceptible. But *R. muelleri and R. bowersi* showed a certain degree of natural resistance to the worm but *R. whiteheadi* and *R. cremoriventer* were highly susceptible and they died within 10 days of infection with 200 infective larvae (Heyneman and Lim, 1965). *T. glis* was observed to die between 15 to 18 days after a heavy infection (Lim, 1970). It is therefore quite probable that the low worm-load in *R. cremoriventer*, *R. whiteheadi* and *T. glis* represented those with light infection in nature that survived and *R. muelleri* and *R. bowersi* showed partial resistance to the parasite.

The present study also recorded new host of *A. malaysiensis* in four species of forest rats, *R. annandalei*, *R. surifer*, *R. cremoriventer*, *R. whiteheadi* and a species of primitive primate, *T. glis*. It is obvious from the results of this observation that *A. malaysiensis* is an indigenous parasite of this country. The fact that human cases have been reported recently in Peninsular Malaysia and Borneo (Watts, 1969 and Bisseru *et al.*, 1972), suggests that the parasite may be pathogenic to man, although in both instances, the authors only assumed that the larval worms recovered in the cerebrospinal fluid in both cases were *A. cantonensis*.

SUMMARY

Angiostrongylus worms recovered from a total of 982 forest animals comprising of six species of wild forest rats and a species of

primitive primate were examined. Comparative studies of the worms with those from the field rats showed that they both belong to the same species of *Angiostrongylus malaysiensis*.

New hosts, *Rattus annandalei*, *R. cremoriventer*, *R. whiteheadi* and *R. surifer*, and a species of primitive primate, *Tupaia glis* of *A. malaysiensis* are reported for the first time.

The low rate of natural infection in the forest animals with the parasite was probably attributed to the scarcity of the intermediate hosts in the forest habitats. Resistance and susceptibility to the parasite of some of these forest animals were discussed.

ACKNOWLEDGEMENTS

The author is grateful to Mr. M. Krishnansamy, for his assistance; to the Director, Dr. R. Bhagwan Singh, Institute for Medical Research, Kuala Lumpur for permission to publish this paper.

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