Jpn. J. Vet Res., 28, 114–121 (1980)

REPORT ON THE PREVALENCE AND EXPERIMENTAL INFECTIONS OF *ANGIOSTRONGYLUS SIAMENSIS* OHBAYASHI, KAMIYA ET BHAIBULAYA, 1979, PARASITIC IN THE MESENTERIC ARTERIES OF RODENTS IN THAILAND*1

Masao KAMIYA, Yuzaburo OKU, Ken KATAKURA, Haruo KAMIYA, Masahi OHBAYASHI, Hisashi ABE*2, Hiroshi SUZUKI*3 and Manoon BHAIBULAYA*4

Department of Parasitology
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

(Received for publication, 26 June, 1980)

Following the discovery of *Angiostrongylus siamensis* OHBAYASHI, KAMIYA et BHAIBULAYA, 1979, which is parasitic in the mesenteric artery of *Rattus sabanus*, the parasite was recovered from 5 rats of 278 micromammals collected from 6 localities in Thailand from July to August, 1978, and in February, 1979: 1 to *R. sabanus*; 1 to *R. berdmorei*; 1 to *R. rattus*; and 2 to *R. surifer*, thus, extending the focus of the study, which had been limited to one locality in Thailand and the host range of the parasite.

The first stage larvae were fed to an experimental intermediate host, *Biomphalaria glabrata*, and the third stage larvae were successfully obtained on the 25th day after exposure. The third stage larvae were inoculated orally into the following laboratory animals: mice (ICR), rats (WKA), cotton rats (*Sigmodon hispidus*) and Mongolian gerbils (*Meriones unguiculatus*). Adult worms were collected from the mesenteric arteries, and partially from the aortae of the respective animals, indicating that this strain of *A. siamensis* had been successfully maintained in the laboratory between the snails, *B. glabrata*, and the small laboratory animals.

INTRODUCTION

Eighteen species of *Angiostrongylus*, including the one inquirendae, have been reported. Among them, only 2 species have so far been known to be causative agents

*1 This study was supported by Overseas Scientific Research Grant No. 304105 and 404304 from the Ministry of Education, Science and Culture, Japan.

*2 Institute of Applied Zoology, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

*3 Department of Virology, Institute for Tropical Medicine, Nagasaki University, Nagasaki 852, Japan

*4 Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok 4, Thailand

of well-known human parasitoses, i. e., eosinophilic menigoencephalitis caused by *A. cantonensis*[2,3,4,6,7,15,17,19,20,21,24] and abdominal granuloma caused by *A. costaricensis*[5,10~14,18,22,23,25].

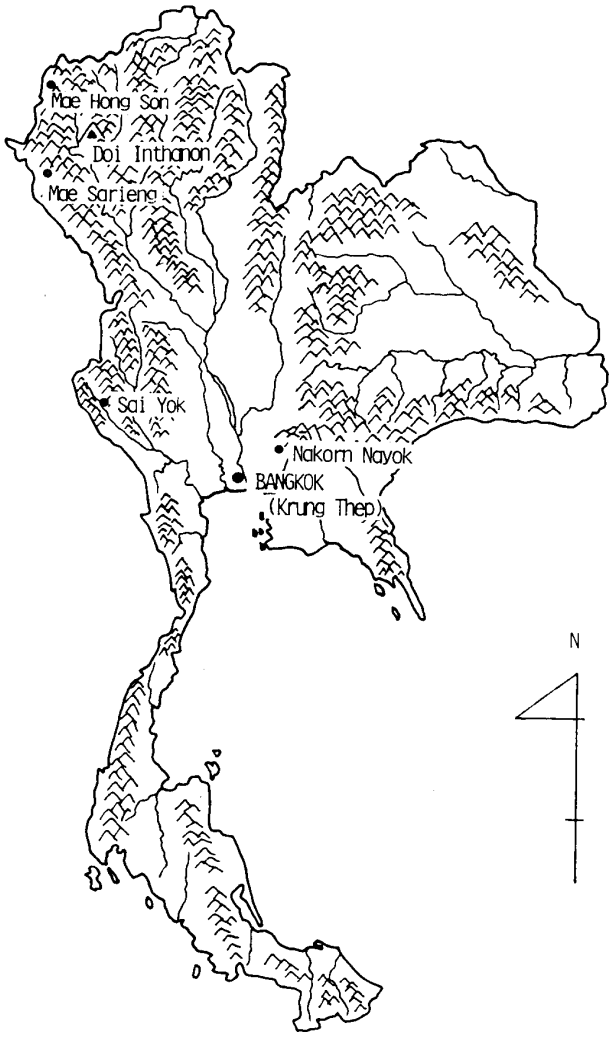
Following a phylogenic study on the helminthic parasite of rodents in Thailand in 1978, a new angiostrongylid nematode, *A. siamensis* OHBAYASHI, KAMIYA et BHAIBULAYA, 1979[16], resembling *A. costaricensis* in its parasitizing habitat, was recovered from the mesenteric arteries of *Rattus sabanus* collected in an evergreen tree forest in Nakorn Nayok, Thailand. Thereafter, the prevalence of *A. siamensis* among micromammals was surveyed from July to August, 1978 and in February, 1979, in various places in Thailand. In addition, we conducted experiments to establish a laboratory strain of the parasite using laboratory animals.

MATERIALS AND METHODS

During the period from July 18 to August 7, 1978, and from February 10 to 24, 1979, micromammals were collected from 6 localities in Thailand, i. e., Bangkok, Sai Yok, Nakorn Nayok, Mae Hongson, Mae Sariang and Doi Inthanon (fig. 1). The animals were mainly captured alive in Sherman traps or in cage traps baited with bananas, sacrificed with chloroform, measured, and skinned. Dr. H. ABE of the Institute of Applied Zoology, Faculty of Agriculture, Hokkaido University, identified the host animals according to procedures he described previously[1]. The abdominal cavity was opened and the mesenteric artery and aorta were carefully examined for the presence of adult worms. The gross appearance of the caecum was noted. The worms were collected with a dissection microscope and preserved in 10% formalin.

In addition, some of the intestinal wall lesions of *R. sabanus*, which contained the first stage larvae, were fed to snails, *Biomphalaria glabrata*, which had been bred in the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Thailand. The snails were kept at 25° to 30°C for 25 days, after which the larvae were freed successfully from the tissues of the snails by digestion in artificial gastric juice and separation by capillary pipette under a dissection microscope. The following animals were used at 6 weeks old for oral inoculations (ranging from 15-40) of the larvae: 3 male ICR mice, 6 male cotton rats, *Sigmodon hispidus*, 6 male WKA rats, and 3 male Mongolian gerbils, *Meriones unguiculatus* (tab. 3). The mice were obtained from the CLEA Breeding Laboratory, Kawasaki, Japan, and the other animals were supplied by the animal laboratory of the Department of Parasitology, Faculty of Veterinary Medicine, Hokkaido University, Japan. The adult worms were recovered mainly from the mesenteric arteries of the respective animals after they had expired.

FIGURE 1 *Survey places in Thailand*



RESULTS

Prevalence in micromammals

A total of 278 micromammals was collected from 6 localities in Thailand. Of the 278, 164 belonged to genus *Rattus*, viz., 5 to *R. berdmorei*; 4 to *R. bukit*; 50 to *R. confucianus*; 27 to *R. exulans*; 30 to *R. rattus*; 5 to *R. sabanus*; and 29 to *R. surifer*, and the remaining 114 comprised the genera *Bandicota*, *Mus*, *Hylopetes*, *Crocidura*, *Tupaia*, *Hipposideros*, *Myotis*, *Hylomys*, *Eothenomys*, *Menetes*, etc. (tab. 1). Table 1 shows the prevalence of *A. siamensis* in rodents from all of the localities. Three female *A. siamensis* worms were collected first from a noisy rat, *R. sabanus* (female, body weight 408 g, head and body 254 mm, tail 368 mm, fore foot 23.5 mm, hind foot 48.5 mm, ear 30.5 mm) in Nakorn Nayok, and it was used to establish the laboratory strain

TABLE 1 *Number of host animals infected with Angiostrongylus siamensis*

SPECIES	PLACE COLLECTED (DATE)							
	Sai Yok Noi (July '78)	Nakorn Nayok (July '78)	Nakorn Nayok (Feb. '79)	Bangkok (July '78)	Mae Hong Son (Aug. '78)	Doi Inthanon (Feb. '79)	Mae Sarieng (Feb. '79)	Total
Rattus berdmorei	—	—	1/3[1)]	—	0/1	—	0/1	1/5
R. bukit	—	0/3	0/1	—	—	—	—	0/4
R. confucianus	—	—	—	—	—	0/50	—	0/50
R. exulans	0/1	—	—	0/1	0/25	—	—	0/27
R. fulvescens	—	—	—	—	—	0/2	—	0/2
R. norvegicus	—	—	—	0/12	—	—	—	0/12
R. rattus	0/2	0/6	1/15	—	0/6	—	0/1	1/30
R. sabanus	—	1/4	0/1	—	—	—	—	1/5
R. surifer	0/2	0/8	2/19	—	—	—	—	2/29
Bandicota indica	—	—	0/3	—	—	—	—	0/3
B. savilei	—	—	0/6	—	—	—	—	0/6
Mus. spp.	0/7	0/1	—	—	—	0/3	—	0/11
Others[2)]	0/6	0/3	0/16[3)]	0/1[4)]	0/50	0/18	—	0/94
Total	0/18	1/25	4/64	0/14	0/82	0/73	0/2	5/278

1) Number positive/number examined
2) Micromammals other than the genera *Rattus*, *Bandicota* and *Mus*: *Crocidura*, *Tupaia*, *Hipposideros*, *Myotis*, *Hylomys*, *Eothenomys*, *Menetes* etc.
3) *Menetes berdmorei* 4) A specimen of *Hylopetes phayrei* obtained at market

TABLE 2 *Measurement of host animals infected with Angiostrongylus siamensis and number of worms collected*

HOST*	DATE OF COLLECTION	SEX	BW	HB	T	FF	HF	E	NO. WORMS COLLECTED	
									♀	♂
			g	mm	mm	mm	mm	mm		
Rattus sabanus	July 22, '78	♀	408	254	368	23.5	48.5	30.5	3	0
R. berdmorei	Feb. 11. '79	♀	138	184	152	17.5	37	23.5	8	6
R. rattus	Feb. 12, '79	♂	90	165	176	14.3	30.5	20.5	1	0
R. surifer	Feb. 13, '79	♂	173	192	198	16.5	38	24	2	2
R. surifer	Feb. 13, '79	♀	160	187	177	17	38	24	0	1

BW: Body weight, HB: head and body, T: tail, FF: fore foot, HF: hind foot, E: ear
* All specimen were captured in Nakorn Nayok, Thailand.

of the parasite. The over-all frequency of *A. siamensis* was 1.8%, although the prevalence was limited to Nakorn Nayok and to 4 species of genus *Rattus* (i. e., 1 out of 3 *R. berdmorei*, 33.3%, 1 out of 21 *R. rattus*, 4.8%, 1 out of 5 *R. sabanus*, 20%, and 2 out of 27 *R. surifer*, 7.4% (tab. 2). Table 2 gives the measurements of the host animals infected with *A. siamensis* and the number of worms collected. Of the 5 rats naturally infected with the parasite, only the first case of *R. sabanus* showed a slightly thickened and hardened edematous intestinal wall, the remaining 4 showed few lesions macroscopically.

Experimental infections in laboratory animals

The third stage larvae were obtained successfully from the *B. glabrata* snails. The first stage larvae were found in the feces of rats and cotton rats from the initial fecal examination at the 29th day after infection; however, at the 34th day, the mice turned to positive. No larvae were collected from the two gerbils, which expired on the 21st and 25th days after infection. Gerbil No. 1, which expired on the 79th day, did not show any larvae because it harboured only the male worm. The adult worms

TABLE 3 *Infectivity of laboratory animals with Angiostrongylus siamensis (dead cases)*

ANIMAL NO.		NO. OF LARVAE INFECTED	DAYS AFTER INFECTION	NO. OF ADULTS RECOVERED ♂ ♀
Mouse	1	15	150	2 (1, 1)
	2	20	94	3 (2, 1)
	3	30	42	11 (6, 5)
Cotton rat	1	15	33	4 (1, 3)
	2	15	33	7 (1, 6)
	3	20	44	5 (1, 4)
	4	20	36	6 (4, 2)
	5	30	32	6 (0, 6)
	6	40	32	8 (2, 6)
Rat	1	15	57	6 (5, 1)
	2	15	53	6 (1, 5)
	3	20	72	4 (1, 3)
	4	20	53	2 (0, 2)
	5	30	42	4 (1, 3)
	6	40	95	6 (3, 3)
Mongolian gerbil	1	15	79	1 (1, 0)
	2	20	21	6 (6, 0)
	3	30	25	4 (2, 2)

were successfully recovered from the mesenteric arteries and aortae of the animals used in the present experiment (tab. 3).

The previously reported description of the male *A. siamensis* worms by OHBAYASHI et al. (1979)[16] was based on that of the worms obtained in this experiment.

DISCUSSION

The discovery of *A. siamensis*, which resembles *A. costaricensis* in its parasitizing behavior of the mesenteric artery in rodents, provided us with significant information on both the phylogenics of rodents and the evolution and distribution of angiostrongylid nematodes in the world. It was also interesting to note that *A. siamensis* may act as an agent of parasitic zoonosis because of its broad range of final hosts, resembling *A. costaricensis*. *A. costaricensis* has been reported to be an agent of abdominal granuloma, especially in children in Costa Rica by MORERA & CÉSPEDES[14]. Although the geographic distribution of the *A. siamensis* in our study was limited to the evergreen tree forest in Nakorn Nayok, our survey and experimental infections increased the known host range of *A. siamensis* to 3 additional species besides *R. sabanus* in the endemic area: *R. berdmorei*, *R. rattus* and *R. surifer*, and by 4 more species in laboratory animals: mice, cotton rats (*Sigmodon hispidus*), rats and gerbils (*Meriones unguiculatus*). It is suggested that *A. siamensis* has a wide range of susceptibility to the host as reported in *A. costaricensis* infection[23].

The prepatent period (earlier than the 29th day) in *A. siamensis* is much shorter than that of *A. cantonensis* and *A. vasorum*; however, because we could not check the initial appearance of first stage larvae in the feces, the exact prepatent period could not be determined.

More detailed information on the growth of *A. siamensis*, the pathological findings of the experimental intermediate host, *B. glabrata*[8,9], and the various experimental animals used as the final hosts will be published separately.

ACKNOWLEDGEMENTS

The authors wish to express their sincere appreciation to Mr. Sanga SABHASRI, Secretary General, the National Research Council, Thailand, for granting the permission to conduct research in Thailand. We also indebted to Prof. Chamlong HARINASUTA, Prof. Suvajra VAJRASHIRA, and Prof. Santasiri SORNMANI, Faculty of Tropical Medicine, Mahidol University for their kind support of this study.

Thanks are also due to Ms. Kosoom KLONGKAMNUARNKARN and Mr. Somchai IMLARP, Department of Tropical Hygiene, Faculty of Tropical Meidcine, Mahidol University, for provision of the materials.

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