

Geographical distribution and incidence of *Angiostrongylus* lungworms (Nematoda: Angiostrongylidae) and their rodent hosts in Thailand

Eamsobhana, P.^{1*}, Yong, H.S.², Prasartvit, A.³, Wanachiwanawin, D.¹ and Boonyong, S.¹

¹Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

²Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

³Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand

*Corresponding author e-mail: praphathip.eam@mahidol.ac.th; darawan.wan@mahidol.ac.th

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Abstract. The rat lungworm *Angiostrongylus cantonensis*, a zoonotic parasite, is known to be responsible for eosinophilic meningitis and meningoencephalitis in humans in many countries worldwide. Another congener *A. malaysiensis* is a potential pathogen. Rodents as natural definitive host of the parasites are abundant and globally widespread. In this study, the prevalence of *Angiostrongylus* infection in wild rats was investigated in twenty-four provinces of Thailand during the period December 2011 to June 2014. Of the 669 wild rats sampled, 46 (6.88%) were infected with *Angiostrongylus* lungworms. The rodents harbouring *A. cantonensis* worms included *Bandicota indica*, *Bandicota savilei*, *Rattus exulans*, *Rattus norvegicus*, *Rattus rattus* complex and *Rattus tiomanicus*, and those harbouring *A. malaysiensis* were *B. savilei*, *Rattus losea*, *R. norvegicus* and *R. rattus* complex. No parasite was recovered from *Maxomys surifer* (n=11), *Mus musculus* (n=1), *Niviventer fulvescens* (n=2), *Rattus argentiventer* (n=4), *Rattus nitidus* (n=3) and *Sundamys muelleri* (n=3). In positive rats, the incidence of infection with *Angiostrongylus* lungworms was variable among host species and provinces. There were also considerable variation in the proportion of male and female worms among rodent hosts and localities. Two hundred and thirty-five of the collected worms were male and 282 were female. The mean worm burden in the positive rats was 11.24 and ranged from 1 to 61. 81.82% (423/517) of the adult worms were morphologically identified as *A. cantonensis*, and 18.18% (94/517) were *A. malaysiensis*. One *R. rattus* from Prachuap Khiri Khan had mixed infection of *A. cantonensis* and *A. malaysiensis* (10 worms of each species). The overall number of male (202) and female (221) *A. cantonensis* worms was not significantly different ($\chi^2 = 0.86$, $0.50 > P > 0.30$). However, the overall number of male (33) and female (61) *A. malaysiensis* worms was significantly different ($\chi^2 = 8.34$, $P < 0.01$). The present study added one new definitive host (*R. tiomanicus*) for *A. cantonensis* and two new definitive hosts (*B. savilei* and *R. losea*) for *A. malaysiensis* in Thailand. Our data update and contribute significantly to existing knowledge of the geographical distribution of *A. cantonensis* in wild rats in Thailand and confirm the occurrence of *A. malaysiensis* throughout the country.

INTRODUCTION

Angiostrongylus cantonensis (Chen, 1935), a metastrongyloid nematode of rodents, was first observed in Canton (Guangzhou), China in the lungs of *Rattus norvegicus* and *Rattus rattus*. It is widely distributed in Southeast Asia, Pacific islands, the Caribbean, Australia, and South and North America (Wang

et al., 2008; 2012). This rat lungworm and the closely related species, *A. mackerrasae* (Bhaibulaya, 1968) and *A. malaysiensis* (Bhaibulaya and Cross, 1971) occur naturally in the pulmonary artery and heart of wild rats. *A. cantonensis* has been documented to cause human eosinophilic meningitis or meningoencephalitis (Wang *et al.*, 2008; 2012; Eamsobhana, 2014). Ocular angio-

strongyliasis and angiostrongyliasis involving the lungs have also been reported (Eamsobhana, 2014).

The life cycle of *A. cantonensis* involves rodent definitive host and mollusk/snail intermediate host (Bhaibulaya, 1975). Man is an accidental host, acquiring the infection by consuming raw or poorly cooked snail meat which harbor the infective larvae of the parasite (Cross, 1987). In nature, many other animals such as land crabs, frogs, freshwater prawns or monitor lizard can serve as paratenic host for the parasite (Cross & Chen, 2007). In Thailand, human cases of eosinophilic meningitis caused by *A. cantonensis* have been reported largely from the northeastern part of the country (Eamsobhana, 2014). The disease is more commonly seen in adults and infection is about twice as common in males than females. Males often eat undercooked snail dishes while drinking alcoholic beverages. In earlier studies, seven species of rodents – *Bandicota indica*, *Bandicota savilei*, *Maxomys surifer*, *Rattus berdmorei*, *Rattus exulans*, *Rattus norvegicus*, and *Rattus rattus* – have been found to be definitive hosts in Thailand (Crook *et al.*, 1968; Harinasuta *et al.*, 1965; Punyagupta *et al.*, 1970).

Angiostrongylus malaysiensis has been found singly or with *A. cantonensis* in *R. norvegicus* and *R. rattus* in Thailand (Bhaibulaya & Techasophonmani, 1972; Jeradit, 1977). However, there is no clinical evidence to suggest that it is associated with human eosinophilic meningitis.

Most studies of the rodent hosts of *A. cantonensis* in Thailand were from the northern and northeastern parts of the country. There are limited reports about *A. cantonensis* infection among rodent hosts in other parts of Thailand (Eamsobhana, 2014). The actual prevalence and distribution of *A. cantonensis* in the country remain unknown. Additionally there are little information on *A. malaysiensis* apart from the studies of Bhaibulaya and Techasophonmani (1972) and Jeradit (1977). We report here the country-wide distribution and incidence of *Angiostrongylus* lungworms and their rodent hosts in Thailand.

MATERIAL AND METHODS

Study areas and rodent collection

Rodents were captured by wire cage traps baited with food in 24 provinces in Thailand (Figure 1). The various species of rodents were anesthetized and identified to species based on keys and illustrations developed by Marshall (1988).

All procedures involving animals were conducted under animal use protocols approved by the Animal Ethical Committee of the Ministry of Public Health, Thailand (approval no. FWA00013622).

Parasitological procedures

Lungs and heart of individual captured rats were removed and placed in a small plastic bag with zipper, stored in ice, and shipped/transported to the Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University for rat lungworm examination. Upon arrival at the Parasitology laboratory, the lungs and heart samples were put in Petri dishes, dissected and sorted under stereomicroscope for the *Angiostrongylus* adult worms. The collected worms were washed in physiological saline and identified morphologically as belonging to *Angiostrongylus* species under light microscope according to existing keys and descriptions (Bhaibulaya, 1979; Bhaibulaya & Cross, 1971; Eamsobhana, 2014). After identification, the worms were kept in absolute alcohol at -70°C for future genomic studies.

RESULTS

A total of 669 wild rats comprising the genera *Bandicota* (2 species), *Rattus* (7 species), *Maxomys* (1 species), *Mus* (1 species), *Sundamys* (1 species) and *Niviventer* (1 species) were caught during the study period (Table 1). Table 2 summarizes the prevalence of infection with *Angiostrongylus* lungworms. No parasite was recovered from *M. surifer* (n=11), *Mus musculus* (n=1), *N. fulvescens* (n=2), *R. argentiventer* (n=4), *R. nitidus* (n=3) and

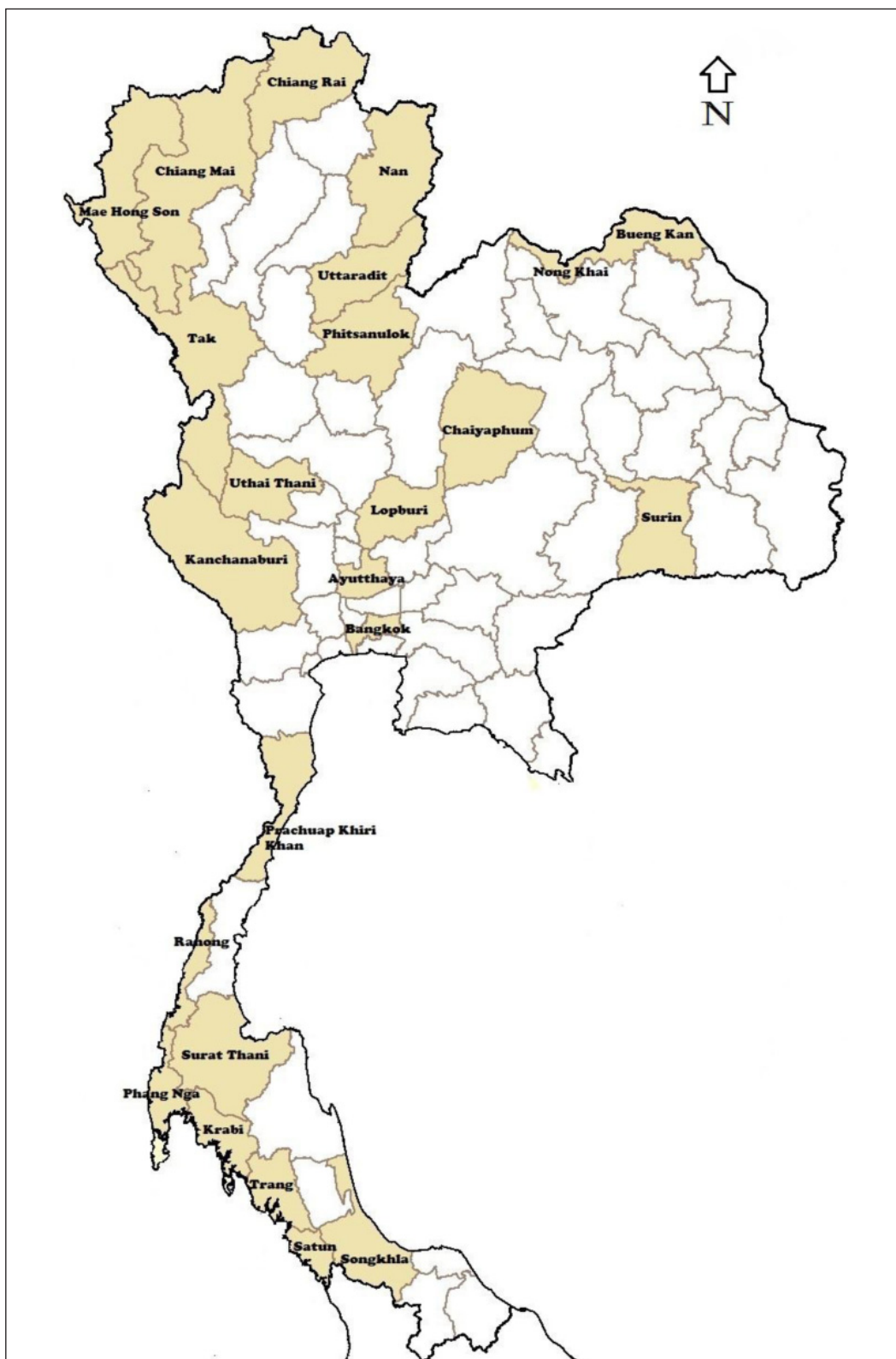


Figure 1. Map of Thailand showing provinces where rodents were collected in this study for *Angiostrongylus* lungworm detection and identification.

Table 1. Rodent species trapped in different provinces of Thailand for study on infection with *Angiostrongylus* worms

Locality: Province/District	Rodent species
North	
Mae Hong Son/Muang	<i>Bandicota indica</i> , <i>Rattus losea</i> , <i>Rattus exulans</i>
Chiang Rai/Muang	<i>Bandicota indica</i> , <i>Rattus exulans</i> , <i>Rattus rattus</i> complex
Chiang Rai/Mae Sai	<i>Rattus rattus</i>
Chiang Mai/Muang	<i>Bandicota indica</i>
Uttharadit/Ban khok	<i>Bandicota indica</i> , <i>Rattus losea</i> , <i>Rattus rattus</i> complex
Nan/Chaloem Phra Kiat	<i>Rattus rattus</i> complex
Central	
Phitsanulok/Muang	<i>Bandicota indica</i> , <i>Bandicota savilei</i> , <i>Mus musculus</i> , <i>Rattus exulans</i> , <i>Rattus rattus</i> complex
Bangkok/Don Muang	<i>Rattus exulans</i> , <i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex
Bangkok/Bangkok Noi	<i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex
Bangkok/Bang Phlat	<i>Rattus exulans</i> , <i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex
Bangkok/Bang Khun Thian	<i>Rattus rattus</i> complex
Bangkok/Bang Bon	<i>Rattus exulans</i> , <i>Rattus norvegicus</i>
Bangkok/Bang Chun	<i>Rattus exulans</i> , <i>Rattus rattus</i> complex
Bangkok/Minburi	<i>Rattus exulans</i> , <i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex
Bangkok/Chatuchak	<i>Rattus exulans</i> , <i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex
Bangkok/Bang Kapi	<i>Rattus norvegicus</i>
Bangkok/Bang Khen	<i>Rattus exulans</i> , <i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex
Ayuthaya/Pachee	<i>Bandicota indica</i> , <i>Bandicota savilei</i> , <i>Rattus argentiventer</i> , <i>Rattus exulans</i> , <i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex
Lopburi/Muang	<i>Bandicota indica</i> , <i>Bandicota savilei</i> , <i>Rattus nitidus</i> , <i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex
UthaiThani/Lan Sak	<i>Maxomys surifer</i> , <i>Rattus rattus</i> complex
Northeast	
Chaiyaphum/Khon Sarn	<i>Niviventer fulvescens huang</i> , <i>Rattus losea</i> , <i>Rattus rattus</i> complex
Surin/Kap choang	<i>Maxomys surifer</i> , <i>Rattus exulans</i>
Nong Khai/Muang	<i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex
Bueng Kan/Muang	<i>Rattus losea</i>
West	
Tak/Mae Sot	<i>Bandicota savilei</i> , <i>Rattus exulans</i> , <i>Rattus rattus</i> complex
Kanchanaburi/Muang	<i>Bandicota indica</i> , <i>Maxomys surifer</i> , <i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex
Prachuap Khiri Khan/Hua Hin	<i>Rattus exulans</i> , <i>Rattus rattus</i> complex
South	
Ranong/Muang	<i>Maxomys surifer</i> , <i>Niviventer fulvescens bukit</i> , <i>Rattus exulans</i> , <i>Rattus rattus</i> complex
Surat Thani/Vibhavadi	<i>Bandicota indica</i> , <i>Bandicota savilei</i> , <i>Rattus rattus</i> , <i>Rattus tiomanicus</i>
Krabi/Khlong Thom	<i>Rattus exulans</i> , <i>Rattus rattus</i> complex
Ranong/Muang	<i>Rattus exulans</i> , <i>Rattus norvegicus</i> , <i>Rattus rattus</i> complex, <i>Rattus tiomanicus</i>
Surat Thani/Phun Phin	<i>Bandicota indica</i> , <i>Rattus exulans</i> , <i>Rattus rattus</i> complex, <i>Rattus tiomanicus</i>
Trang/Kantang	<i>Rattus exulans</i> , <i>Rattus rattus</i> complex, <i>Rattus tiomanicus</i>
Song Khla/Singhanakhon	<i>Bandicota indica</i> , <i>Rattus rattus</i> complex
Song Khla/Nathawi	<i>Rattus exulans</i> , <i>Rattus rattus</i> complex, <i>Rattus tiomanicus</i>
Satun/Muang	<i>Rattus exulans</i> , <i>Rattus rattus</i> complex, <i>Rattus tiomanicus</i>
Satun/ Khuan Don	<i>Rattus exulans</i> , <i>Rattus rattus</i> complex, <i>Rattus tiomanicus</i>
Phang Nga/Muang	<i>Rattus rattus</i> complex, <i>Sundamys muelleri</i>

Table 2. Incidence of infection with *Angiostrongylus* lungworms in rodent definitive hosts in various provinces in Thailand (PLEASE USE COMBINED TABLE 2)

Host species	Locality	Incidence (% infected)	No. worms				Date	
			<i>A. cantonensis</i>		<i>A. malaysiensis</i>			
			♂	♀	♂	♀		
<i>Rattus norvegicus</i>	Bangkok	0/1					Jun 2014	
		0/78					Dec 2011	
		2/17 (11.8)	23	24			Dec 2011	
		0/22					Jan 2012	
	Ayuthaya	0/9					Dec 2011	
		Lopburi	2/43 (4.7)	2	0			Jan 2012
	Nong Khai	1/6 (16.7)	5	10	4	6		Feb 2014
		Kanchanaburi	1/2 (50)			1	0	
Ranong	1/6 (16.7)			1	0		Mar 2014	
<i>Rattus rattus</i> complex	Chiang Rai	2/2 (100)	20	4			Jan 2014	
	Uttharadit	0/2					Jan 2014	
	Nan	0/2					Jan 2014	
	Phitsanulok	1/4 (25)	4	7			Jun 2014	
	Bangkok	1/6 (16.7)			0	2		Jun 2014
		0/35						Dec 2011
		0/2						Jan 2012
	Ayuthaya	0/8						Dec 2011
	Lopburi	0/9						Jan 2012
	Uthai Thani	0/7						Jan 2013
	Chaiyaphum	0/3						Dec 2013
	Nong Khai	0/4						Feb 2014
	Tak	0/2						Jan 2013
	Kanchanaburi	0/8						Mar 2014
	Prachuap Khiri Khan	1/5 (20)	5	5	3	7		Mar 2014
	Ranong	1/31 (3.2)	1	0				Feb 2013
		0/9						Mar 2014
	Surat Thani	0/5						Feb 2013
		0/6						Mar 2014
	Krabi	0/8						Feb 2013
	Phang Nga	1/12 (8.3)	1	0				May 2013
		1/12 (8.3)			3	0		
	Trang	2/16 (12.5)	1	13				Apr 2014
		1/16 (6.3)			0	10		
	Songkhla	0/8						Apr 2014
	Satun	0/23						Apr 2014
		1/34			1	2		Apr 2014
	<i>Rattus tiomanicus</i>	Ranong	0/9					Mar 2014
Surat Thani		0/3					Feb 2013	
		0/1					Mar 2014	
Trang		1/15 (6.7)	0	2			Apr 2014	
Songkhla		0/6					Apr 2014	
Satun		0/14					Apr 2014	
<i>Sundamys mulleri</i>	Phang Nga	0/3					May 2013	
Total		46/669 (6.9)	202	221	33	61		

S. muelleri (n=3). Two hundred and thirty-five male and 282 female worms were recovered from infected rodents. The mean worm burden in the positive rats was 11.24 and ranged from 1 to 61. 81.82% (423/517) of

the adult worms were morphologically identified as *A. cantonensis*, and 18.18% (94/517) were *A. malaysiensis* (Figures 2-5).



Figure 2. Tail tip of male *A. cantonensis* showing copulatory bursa with separation of ventro-ventral (vv) and latero-ventral (lv) rays at about the distal one-third of the common trunk (arrow). pl; postero-lateral ray, ml; medio-lateral ray, al; antero-lateral ray, ed; external dorsal ray.



Figure 3. Tail tip of male *A. malaysiensis* showing copulatory bursa with separation of ventro-ventral (vv) and latero-ventral (lv) rays at about the distal two-thirds of the common trunk (arrow). pl; postero-lateral ray, ml; medio-lateral ray, al; antero-lateral ray, ed; external dorsal ray.

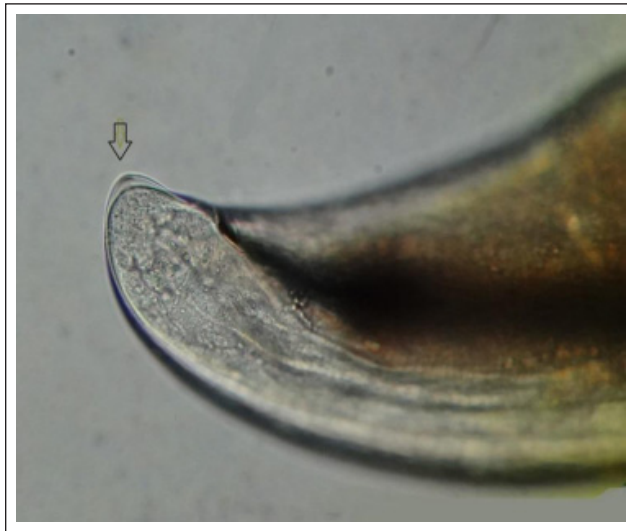


Figure 4. Tail tip of female *A. cantonensis* has no inner projection (arrow).



Figure 5. Tail tip of female *A. malaysiensis* showing inner minute projection (arrow).

DISCUSSION

The prevalence of *A. cantonensis* infection in seven species of murid rodents (*B. indica*, *B. savilei*, *Berymys berdmorei*, *M. surifer*, *R. exulans*, *R. norvegicus* and *R. rattus* complex) has been reported for Bangkok, Thonburi, Ubon Ratchathani, Udon Thani, Nakhon Ratchasima, Phitsanulok, Nan,

Chiang Mai and five provinces in northeast Thailand (Eamsobhana, 2014).

When first reported in Thailand, 2.9% of the 309 rats from Bangkok, Thonburi and some provinces from the northeast, were infected with *A. cantonensis* (Harinasuta *et al.*, 1965). In the 1960s, 1.2% of 1,736 rats from 13 provinces were positive for the infection, comprising 4.1% of *B. indica*, 0.7%

M. surifer, 1.3% *R. exulans* and 1.2% *R. rattus* (Punyagupta *et al.*, 1970). The infection rate ranged from 0.5% of 213 rats in Ubon Ratchathani to 9% of 65 rats in Nan, with 1.8% of 275 rats in Chiang Mai, 1.8% of 340 rats in Udon Thani and 4.3% of 115 rats in Nakhon Ratchasima (Punyagupta *et al.*, 1970).

During the last two decades, the prevalence of *A. cantonensis* infection in five provinces in northeast Thailand was 3.8% (2/52) of *R. norvegicus* and 1.4% (1/69) of *B. indica* (Pipitgool *et al.*, 1997); however 151 individuals of *R. rattus* were not infected. During May to August 1995 in Chiang Mai, some 37% of the rats (16 *R. norvegicus* and 17 *R. rattus*) were infected (Namue & Wongsawad, 1997). This finding differed from the 1968 report of 7.8% infection in rats in Chiang Mai (Tanthajamroon, 1968). A more recent survey of *A. cantonensis* in 62 wild rats (*R. argentiventer*, *R. rattus*, *B. indica*, and *B. savilei*) in Phitsanulok province, revealed a prevalence of 1.61% (1/62 rats); the single positive rat was *R. rattus* (Vitta *et al.*, 2011).

Our present study covered 5 provinces in the North, 5 provinces in Central, 4 provinces in Northeast, 3 provinces in West, and 7 provinces in South Thailand (Table 1, Figure 1). The results indicate the occurrence of *A. cantonensis* lungworms in the North (Chiang Rai, and Chiang Mai), Central (Phitsanulok, Bangkok), Northeast (Nong Khai), West (Tak, Prachuap Khiri Khan) and South Thailand (Ranong, Surat Thani, Trang, Song Khla, and Phang Nga) (Table 2). *Angiostrongylus malaysiensis* were also found in the North (Mae Hong Son), Central (Bangkok), West (Tak, Kanchanaburi, and Prachuap Khiri Khan), Northeast (Nong Khai) and South (Ranong, Phang Nga, Trang, and Satun). Two new definitive rodent hosts were identified – *R. losea* for *A. malaysiensis* in Mae Hong Son and *R. tiomanicus* for *A. cantonensis* in Trang (Table 2).

In positive rats, the incidence of infection with *A. cantonensis* lungworms was variable among provinces – 14.3% (Songkhla) to 100% (Chiang Rai) in *B. bandicota*; 16.7% (Phitsanulok) to 72.2% (Tak) in *B. savilei*; 33.3% (Bangkok) to 100% (Tak) in *R. exulans*; 4.7% (Lopburi) to 33.3% (Nong Khai) in

R. norvegicus; and 3.2% (Ranong) to 100% (Chiang Rai) in *R. rattus* complex (Table 2). The prevalence of infection with *A. cantonensis* also differed among host species in the same locality, for example, in Phitsanulok it was 16.7% for *B. savilei*, 40% for *R. exulans* and 25% for *R. rattus* complex (Table 2).

The incidence of infection with *A. malaysiensis* also varied among host species and localities – 5.56% for *B. savilei* in Tak; 100% (1 rat) for *R. losea* in Mae Hong Son; 16.7% for *R. norvegicus* in Nong Khai; and for *R. rattus* complex 16.7% in Bangkok, 20% in Prachuap Khiri Khan, 8.3% in Phang Nga and 6.3% in Trang (Table 2).

There were considerable variation in the proportion of male and female worms among rodent hosts and localities (Table 2). Significantly more *A. cantonensis* male worms were found in *B. indica* in Chiang Rai and Chiang Mai (55 ♂♂, 25 ♀♀; $\chi^2 = 11.25$, $P < 0.001$). The single rat in Chiang Rai harbored 61 worms (38 ♂♂, 23 ♀♀). In contrast, *B. savilei* in Tak harbored significantly more female *A. cantonensis* worms (61 ♂♂, 107 ♀♀; $\chi^2 = 12.60$, $P < 0.001$). However, the proportion of male *A. malaysiensis* worms was significantly higher in *B. savilei* in Tak (20 ♂♂, 7 ♀♀; $\chi^2 = 6.26$, $P < 0.05$). In Prachuap Khiri Khan, there were significantly more female *A. malaysiensis* worms (4 ♂♂, 7 ♀♀) in the black rat (Table 2). Only female *A. malaysiensis* worms were recovered from *R. losea* in Mae Hong Son (27 worms) and *R. rattus* complex (10 worms) in Trang (Table 2). Overall, there were slightly more *A. cantonensis* female worms recovered from the definitive hosts, but not significantly different (202 ♂♂, 221 ♀♀; $\chi^2 = 0.86$, $0.50 > P > 0.30$). In contrast, there were significantly more female *A. malaysiensis* worms (33 ♂♂, 61 ♀♀; $\chi^2 = 8.34$, $P < 0.01$).

The present study added 9 new localities for *A. cantonensis* (Mae Hong Son in the north; Tak, Kanchanaburi and Prachuap Khiri Khan in the west; Nong Khai in the northeast; and Ranong, Phang Nga, Trang and Satun in the south) and two new definitive hosts (*B. savilei* and *R. losea*) for *A. malaysiensis* in Thailand, indicating its wide occurrence in

the country. A black rat (*R. rattus* complex) in Prachuap Khiri Khan had mixed infection of *A. cantonensis* and *A. malaysiensis* (Table 2). Morphological identification of *A. malaysiensis* was confirmed by molecular markers (unpublished data). This finding indicates that care should be taken to identify the *Angiostrongylus* lungworms.

In summary, we report the country-wide occurrence of *Angiostrongylus* lungworms in Thailand and the incidence of infection in various rodent definitive hosts. The dataset will prove useful for future epidemiological and other biomedical studies.

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