# PHYLOGENY OF *ANGIOSTRONGYLUS CANTONENSIS* IN THAILAND BASED ON CYTOCHROME C OXIDASE SUBUNIT I GENE SEQUENCE

Apichat Vitta[1,2,3], Narongrit Srisongcram[1], Jittranuch Thiproaj[1], Anucha Wongma[1], Wilaiwan Polsut[1], Chamaiporn Fukruksa[1], Thatcha Yimthin[1], Bandid Mangkit[4], Aunchalee Thanwisai[1,2,3] and Paron Dekumyoy[5]

[1]Department of Microbiology and Parasitology, Faculty of Medical Science, [2]Center of Excellence in Medical Biotechnology (CEMB), [3]Center of Excellence for Biodiversity Naresuan University, Phitsanulok; [4]Department of Veterinary Technology, Faculty of Veterinary Technology, Kasetsart University, Bangkok; [5]Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

**Abstract.** *Angiostrongylus cantonensis* is an emerging infectious agent causing eosinophilic meningitis or meningoencephalitis in humans with clinical manifestation of severe headache. Molecular genetic studies on classification and phylogeny of *A. cantonensis* in Thailand are limited. This study surveyed *A. cantonensis* larvae prevalence in natural intermediate hosts across Thailand and analyzed their phylogenetic relationships. A total of 14,032 freshwater and land snails were collected from 19 provinces of Thailand. None of *Filopaludina* sp, *Pomacea* sp, and *Cyclophorus* sp were infected with *Angiostrongylus* larvae, whereas *Achatina fulica*, *Cryptozona siamensis*, and *Megaustenia siamensis* collected from Kalasin, Kamphaeng Phet, Phetchabun, Phitsanulok, and Tak Provinces were infected, with *C. siamensis* being the common intermediate host. Based on morphology, larvae isolated from 11 samples of these naturally infected snails preliminarily were identified as *A. cantonensis.* Comparison of partial nucleotide sequences of cytochrome c oxidase subunit I gene revealed that four sequences are identical to *A. cantonensis* haplotype ac4 from Bangkok and the other seven to that of *A. cantonensis* isolate AC Thai, indicating two independent lineages of *A. cantonensis* in Thailand.

**Keywords:** *Angiostrongylus cantonensis*, cytochrome c oxidase subunit I gene, phylogeny, snail, Thailand

## INTRODUCTION

*Angiostrongylus cantonensis* (rat lungworm) is a zoonotic nematode belonging to the superfamily Metastrongylidae and family Angiostrongylidae (Wang *et al*, 2008). *A. cantonensis* was first found in *Rattus* in China (Chen, 1935). At present, this worm is globally present, particularly in Southeast Asia and the Pacific Islands (Eamsobhana, 2006). Its life cycle requires gastropods as intermediate hosts and rodents as definitive hosts (Eamsobhana, 2006). Larvae have been found in diverse

Correspondence: Apichat Vitta, Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand.
Tel: +66 (0) 55 964653; Fax: +66 (0) 55 964770
E-mail apichatv@nu.ac.th

families of mollusk intermediate hosts and adult worms reside in pulmonary arteries and right ventricle of rodent definitive hosts.

Humans are an accidental host, in whom immature worms are not often found in infected organs although over 2,800 cases of human angiostrongyliasis have been recorded from some 30 countries (Wang *et al*, 2008). Nevertheless, *A. cantonensis* is considered as a primary causative agent of eosinophilic meningitis or meningoencephalitis in humans (Eamsobhana, 2006). Recently, this worm has been recognized as being an emerging infectious agent in North and South America, being found in both natural hosts and humans (Maldonado *et al*, 2010; Thiengo *et al*, 2010; Espírito-Santo *et al*, 2013; Teem *et al*, 2013). Transmission of *A. cantonensis* corresponds to the dispersal of intermediate and definitive hosts. Invasive snails and rodents are implicated in an increase of the distribution of *A. cantonensis* in China (Lv *et al*, 2011; Yang *et al*, 2013), Japan (Tokiwa *et al*, 2013) and Brazil (Graeff-Teixeira, 2007).

At least four species of the superfamily Metastrongylidae, namely, *A. cantonensis*, *A. malaysiensis*, *A. siamensis*, and *Thaistrongylus harinasuti*, have been recorded in Thailand (Chen, 1935; Bhaibulaya and Cross, 1971; Ohbayashi *et al*, 1979). Only *A. cantonensis* is the cause of angiostrongyliasis in the country, the first being documented by Khwanmitra *et al* (1957), and since then, over 2,000 cases of human angiostrongyliasis have been reported from all areas of Thailand, but especially in the northeast region of the country where snails are commonly eaten (Khwanmitra *et al*, 1957; Tantibhedyangur, 1963; Benjapongse, 1964; Buranasin *et al*, 1965; Chularerk and Suyarnsethakorn, 1965; Jittayasothorn *et al*, 1965; Punyagupta, 1965; Hongladarom and Indarakoses, 1966; Tangchai *et al*, 1967; Bunnag *et al*, 1969; Punyagupta *et al*, 1970; Nitidandhaprabhas *et al*, 1975; Panamonta, 1985; Witoonpanich *et al*, 1991; Laopornpichayanuwat, 2000; Sawanyawisuth *et al*, 2007).

In view of the number of reported cases of human angiostrongyliasis with eosinophilic meningitis, surveys of the natural hosts of this worm have been conducted. In the early studies, a high prevalence (36.4-94.4%) of this rat lungworm in *Achatina fulica* was reported (Harinasuta *et al*, 1965; Setasubun *et al*, 1968; Pipitgool *et al*, 1997) while a lower prevalence (7.55-12.38%) was reported from recent studies (Tesana *et al*, 2009; Vitta *et al*, 2011). Recently, *Cryptozona siamensis* was identified as a new intermediate host for *A. cantonensis* in Thailand (Vitta *et al*, 2016). Prevalence of *A. cantonensis* in rodent hosts was reported to range from 1.4% to 42.1% from several parts of Thailand (Setasubun *et al*, 1968; Namue and Wongsawad, 1997; Pipitgool *et al*, 1997; Vitta *et al*, 2011).

Use of molecular genetics in identification and analysis of phylogenetic relationships of *A. cantonensis* is limited. Small subunit (SSU) ribosomal (r)DNA has been used for identification of *A. cantonensis* in Japan (Tokiwa *et al*, 2012). However, cytochrome c oxidase subunit I (*co1*) is employed for study of phylogenetic relationships among closely related species (Jefferies *et al*, 2009; Eamsobhana *et al*, 2010a; Foronda *et al*, 2010; Monte *et al*, 2012; Tokiwa *et al*, 2012; Lee *et al*, 2014). Thus, the latter technique was adopted to survey *A. cantonensis* in natural intermediate hosts across Thailand. This study provides a basic knowledge of the genetic diversity and phylogeny related to classification of isolates of *A. cantonensis* in Thailand.

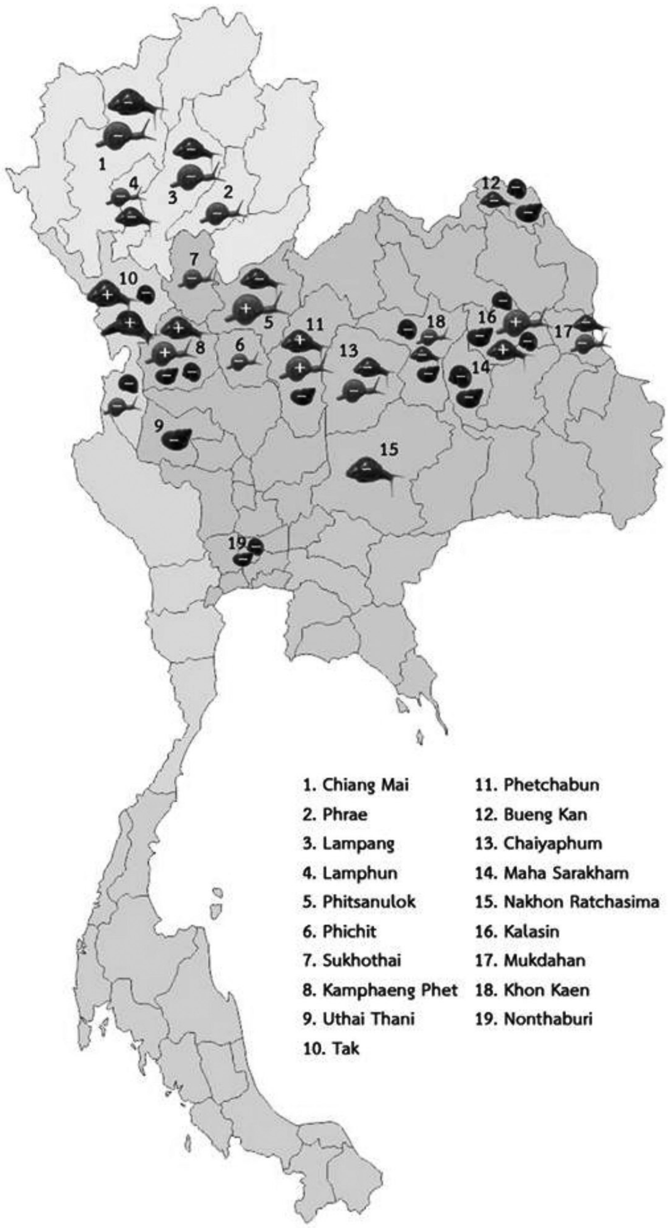


Fig 1–Map of snail sampling provinces across Thailand. Snail with (-) indicates snail negative and with (+) positive for *Angiostrongylus* larvae. Intensity of *Angiostrongylus* larvae was 1.08 and 0.07 in *A. fulica* and *C. siamensis* collected from Phetchabun Province, 1.65 and 0.07 in *A. fulica* and *C. siamensis* from Kalasin Province, 4.33 and 0.01 in *A. fulica* and *M. siamensis* from Tak Province, 0.07 and 0.005 in *A. fulica* and *C. siamensis* collected from Phitsanulok Province, and 0.1 and 23 in *A. fulica* and *C. siamensis* from Kamphaeng Phet Province.

## MATERIALS AND METHODS

### Collection and isolation of *Angiostrongylus* from snails

Fresh water snails (*Filopaludina* sp and *Pomacea* sp) and land snails (*Achatina fulica*, *Cryptozona siamensis*, *Cyclophorus* spp, *Megaustenia siamensis*) were collected between July 2012 and January 2014 from 19 provinces of Thailand (Bueng Kan, Chaiyaphum, Chiang Mai, Kalasin, Kamphaeng Phet, Khon Kaen, Lampang, Lamphun, Maha Sarakham, Mukdahan, Nakhon Ratchasima, Nonthaburi, Phetchabun, Phichit, Phitsanulok, Phrae, Sukhothai, Tak, and Uthai Thani) (Fig 1). Snails were identified at genus and species levels by external shell morphology according to Brandt (1974), Upatham *et al* (1983) and Panha (1996). Snails were digested with 0.7% pepsin solution (Acros Organics, Geel, Belgium) for 1-2 hours at 37°C with shaking. Baermann's technique was used for isolating infective larvae (3$^{rd}$ stage larvae) of *A. cantonensis* (Vitta *et al*, 2016). In brief, a glass funnel joined with a piece of rubber tubing was used as the Baermann apparatus. A wire mesh covered with several layers of gauze was placed at the bottom of the funnel, and with the outlet closed, digested snail content was poured into the funnel until the fluid level made contact the wire mesh. In order to allow *Angiostrongylus* larvae to migrate into the rubber tubing, the apparatus was left standing for 30-60 minutes. At the end of this period, the rubber tubing was opened allowing the fluid containing enriched larvae to be released into a petri dish. Larvae were collected under a stereomicroscope and identified as previously described (Eamsobhana, 2006) and stored at -20°C until used.

**Amplification and sequencing of *A. cantonensis* cytochrome c oxidase subunit I gene (*co1*)**

Genomic DNA from larvae was extracted as previously described (Vitta *et al*, 2016). In brief, 10-50 larvae were put in 5 μl of ATL buffer (Qiagen, Hilden, Germany), grounded and 45 μl of ATL buffer were added. The suspension was vortexed vigorously for one minute, kept at -20°C for 1 hour, and then digested with 15 μl of 10 mg/ml proteinase K at 65°C overnight and at 95°C for 1 hour. The solution was centrifuged at 13,500*g* for 2 minutes and the supernatant was added with 1/10 volume of 5 M NaCl followed by 2 volumes of cold absolute ethanol and then centrifuged at 13,500*g* for 30 minutes. The pellet was washed with 100 μl of 70% ethanol, air dried, dissolved in 15 μl of distilled water and stored at -20°C until used. Primers used were CO1_F (5′ TAAAGAAAGAACATAATGAAAATG 3′) and CO1_R (5′ TTTTTTGGGCATCCTGAGGTTTAT 3′) (Jefferies *et al*, 2009). PCR was performed in 30-μl mixture containing 3 μl of 10X buffer, 4.2 μl of 25 mM $MgCl_2$, 0.6 μl of 200 mM dNTPs, 1.2 μl of 5 μM each primer, 0.6 μl of 100 U Taq DNA polymerase (Sigma, St Louis, MO), 11.7 μl of distilled water and 7.5 μl of DNA template (Vitta *et al*, 2016). Thermocycling was conducted in an Applied Biosystems thermal cycler (Life Technologies, Carlsbad, CA) as follows: 94°C for 5 minutes; followed by 30 cycles of 94°C for 1 minute, 55°C for 30 seconds, and 72°C for 1 minute; with a final heating at 72°C for 5 minutes. Amplicons (450 bp) were analyzed by 1.2% agarose gel-electrophoresis and ethidium bromide-stained bands were visualized and photographed under UV light. Amplicons were purified using a Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, New Taipei City, Taiwan) and sequenced (Macrogen, Seoul, Korea).

**Phylogenetic analysis**

Sequences were edited using SegManII software (DNASTAR, Madison, WI). Sequences of 263-265 bp of *Angiostrongylus co1* from 11 samples were analyzed with ClustalW (Thompson *et al*, 1994) and MEGA ver. 5.0 (Tamura *et al*, 2011) softwares. BLASTN search against known *Angiostrongylus* spp sequences was performed (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Neighbor joining phylogenetic trees were constructed using Kimura-2-parameter model employing MEGA software version 5.05. Bootstrap analysis was performed with 1,000 datasets. Sequences were deposited with GenBank, accession nos. KU934237 - KU934247.

## RESULTS

**Survey of *Angiostrongylus* larvae in intermediate hosts**

A total of 14,032 fresh water and land snails were collected from 19 above mentioned provinces in Thailand. Eleven collected samples of snails from Kamphaeng Phet, Kalasin, Phetchabun, Phitsanulok, and Tak Provinces were positive for *Angiostrongylus* larvae (Fig 1). The highest intensity of *Angiostrongylus* larvae was found in *Cryptozona siamensis* collected from Kamphaeng Phet Province. Preliminary identification of *A. cantonensis* 3rd stage larvae (Fig 2) was made by morphological observation under a light microscope.

Fig 2–Third stage larva of *A. cantonensis* isolated from *Cryptozona siamensis* collected from Kamphaeng Phet Province, Thailand. It is characterized by two chitinous rods at the anterior end (arrow) and a cone-shaped, slightly curved and pointed tail (arrow head) (40x magnification under light microscope).

**Molecular identification and phylogeny analysis**

PCR amplication of *co1* from the 11 putative *Angiostrongylus* 3rd stage larvae generated the expected 450 bp amplicons (data not shown). A neighbor joining tree constructed from the 11 *Angiostrongylus* sequences together with sequences from GenBank database revealed the presence of two main branches (Fig 3). Group I contained one sequence of *A. cantonensis* isolated from Kamphaeng Phet Province, two sequences from Phetchabun Province and one sequence from Tak Province, and included a sequence belonging to *A. cantonensis* haplotype ac4 from Bangkok accession no. AB684368. Group II contained two *A. cantonensis* sequences from Kalasin Province, two from Kamphaeng Phet Province, one sequence from Phitsanulok Province, and two sequences from Tak Province, and included a sequence from Thai *A. cantonensis* accession no. KT186242.

## DISCUSSION

Environment in Asian countries is suitable for development of *A. cantonensis* intermediate and definitive hosts, such as rat, slug and snail. High susceptibility of both types of hosts is considered to be an important factor in the widespread distribution of *A. cantonensis* throughout these areas. In the present study, *A. cantonensis* larvae were identified positively from terrestrial snails *Achatina fulica* and *Cryptozona siamensis* and, although *Angiostrongylus* larvae morphologically identical to *A. cantonensis* were collected from *Megaustenia siamensis* (a new observation), their low numbers precluded positive identification from *co1* sequence. *C. siamensis* was the most common species for hosting *A. cantonensis* in Thailand. Previous studies reported high infection rates of *A. cantonensis* in edible snails from Bangkok and several provinces in northeast Thailand: 90.5% of *Achatina fulica*, 21.7% of *Pila gracilis* and 20% of *P. turbinnis* (Harinasuta *et al*, 1965). In a later survey of natural infections, *A. cantonensis* in mollusks from various parts of Thailand reported incidence rate in *A. fulica*, *P. turbinnis* and *P. gracilis* of 94.4%, 5.71% and 4.13%, respectively, a marked reduction in the two latter species (Setasubun *et al*, 1968). Pipitgool *et al* (1997) also reported the prevalence of

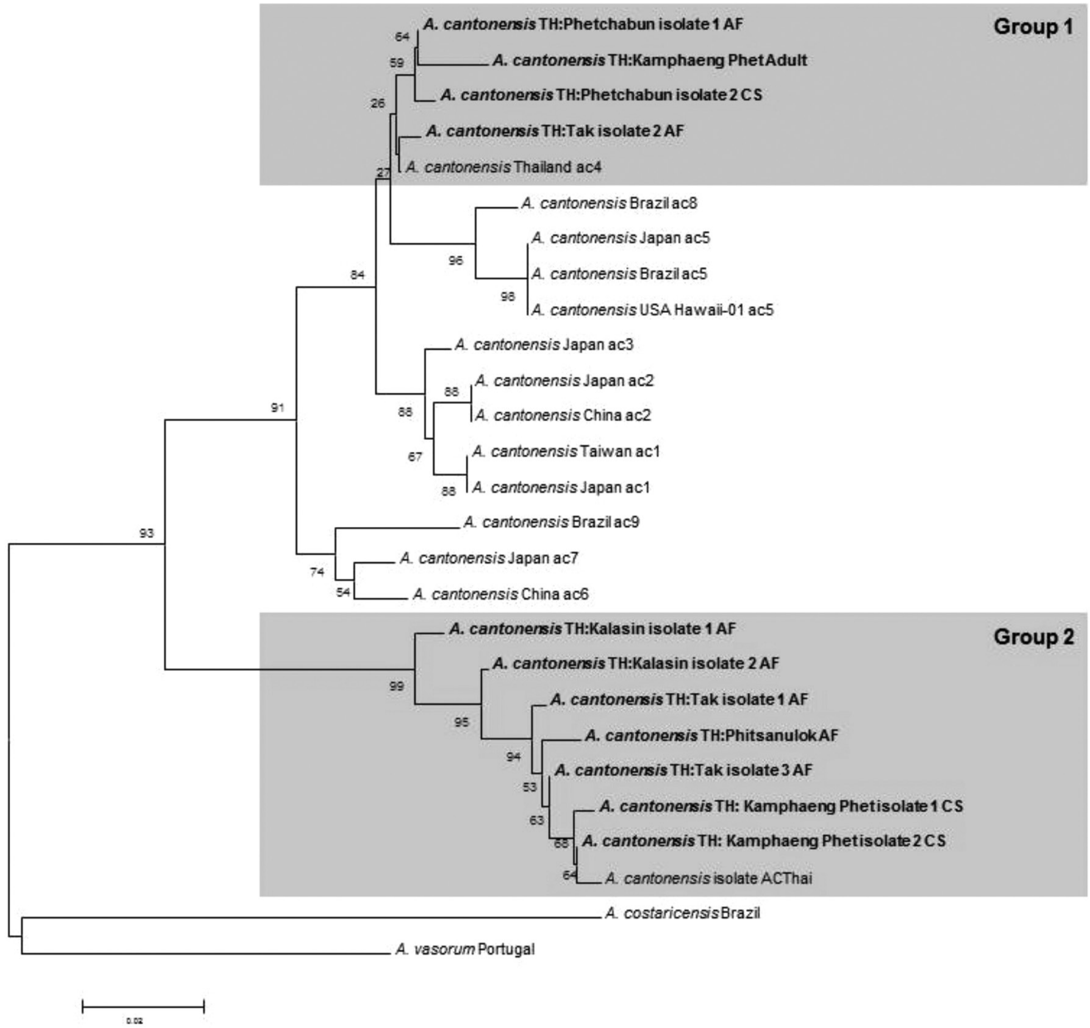


Fig 3–Neighbor joining tree based on 263-265 bp *Angiostrongylus co1* fragment of 11 sequences (bold font) from Thailand together with *A. cantonensis* (regular font) sequences from GenBank database. Bootstrap values are based on 1,000 replicates. *A. costaricensis* and *A. vasorum* are used as out-group. AF, *Achatina fulica*; CS, *Cryptozona siamensis*.

*A. cantonensis* in *Pila polita* and *A. fulica* to be 0.9% and 36.4%, respectively, whereas that of *P. ampullacea* was negative for larval infection. Tesana *et al* (2009) reported most of collected snails have low or no infection but a few snails a high infectivity of *Pila polita*. This is consistent with our findings that none of *Filopaludina* sp, *Pomacea* sp and *Cyclophorus* spp were infected with *A. cantonensis*.

In this study, *A. cantonensis* was divided into two groups based on partial *co1* sequence. This may be due to multiple origins of *A. cantonensis* introduced into Thailand via human transportation. Studies have shown multiple lineages of *A. cantonensis* across the Pacific region (Monte *et al*, 2012; Tokiwa *et al*, 2012). In addition to *co1*, other genetic markers employed in *A. cantonensis* classification and phylogeny have included a 66 kDa protein gene (Eamsobhana *et al*, 2010b), internal transcribed spacers (Jefferies *et al*, 2009; Foronda *et al*, 2010; Liu *et al*, 2011; Lee *et al*, 2014), small subunit ribosomal DNA (Fontanilla and Wade, 2008; Eamsobhana *et al*, 2015), and cytochrome b gene (Dusitsittipon *et al*, 2015; Yong *et al*, 2015). However, *co1* is considered as one of the more powerful markers for differentiating closely related *Angiostrongylus* species and has proven useful in the study of phylogenetic relationships among *A. cantonensis* strains (Eamsobhana *et al*, 2010a; Monte *et al*, 2012; Tokiwa *et al*, 2012).

In summary, *A. cantonensis* from natural intermediate hosts, the terrestrial snails, *A. fulica* and *C. siamensis*, were found at low infection levels in diverse areas of Thailand. In addition, molecular phylogeny revealed two independent origins of *A. cantonensis* in Thailand.

## ACKNOWLEDGEMENTS

The authors thank The Thailand Research Fund, the Commission on Higher Education, Thailand and Naresun University for support (grant no. MRG5580044); the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University for provision of facilities; and Mr Roy Irvine Morien, Naresuan University Language Centre (NULC), Naresuan University for assistance in the English of the manuscript.

## REFERENCES

Benjapongse W. Meningo-encephalomyelitis with eosinophilia in cerebrospinal fluid. *Vejasarn Med J* 1964; 13: 173-84.

Bhaibulaya M, Cross JH. *Angiostrongylus malaysiensis* (Nematode: Metastrongylidae), a new species of rat lung-worm from Malaysia. *Southeast Asian J Trop Med Public Health* 1971; 2: 527-33.

Brandt RAM. The non-marine aquatic mollusca of Thailand. *Arch Molluskenkd* 1974; 105: 1-423.

Bunnag T, Benjapongse W, Noeypatimanond S, Punyagupta S. The recovery of *Angiostrongylus cantonensis* in the cerebrospinal fluid of a case of eosinophilic meningitis. *J Med Assoc Thai* 1969; 52: 665-72.

Buranasin P, Manunpichu K, Tulyaluk P, Settachan D, Juttijudata P, Punyagupta S. The preliminary study of eosinophilic meningitis in Nakornrajsima hospital and review of 50 cases. *Vejasarn Med J* 1965; 14: 1-13.

Chen HT. Un nouveau nematode pulmonaire, *Pulmonema cantonensis* n.g., n. sp. des rats de Canton. *Ann Parasitol Hum Comp* 1935; 13: 312-17.

Chularerk P, Suyarnsethakorn P. *Angiostrongylus cantonensis* (Chen) as a possible cause of a outbreak of meningo-encephalitis in the 4th military circle of the Royal Thai Army. *Royal Thai Army Med J* 1965; 18: 623-30.

Dusitsittipon S, Thaenkham U, Watthanakulpanich D, Adisakwattana P, Komalamisra C. Genetic differences in the rat lungworm, *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae), in Thailand. *J Helminthol* 2015; 89: 545-51.

Eamsobhana P. The rat lungworm *Parastrongylus (=Angiostrongylus) cantonensis*: parasitology, immunology, eosinophilic meningitis, epidemiology and laboratory diagnosis. Bangkok: Wankaew (IQ) Book Center, 2006: 156 pp.

Eamsobhana P, Lim PE, Solano G, Zhang H, Gan X, Yong HS. Molecular differentiation of Angiostrongylus taxa (Nematoda: Angiostrongylidae) by cytochrome c oxidase subunit I (COI) gene sequences. *Acta Trop* 2010a; 116: 152-6.

Eamsobhana P, Lim PE, Yong HS. Phylogenetics and systematics of *Angiostrongylus* lungworms and related taxa (Nematoda: Metastrongyloidea) inferred from the nuclear small subunit (SSU) ribosomal DNA sequences. *J Helminthol* 2015; 89: 317-25.

Eamsobhana P, Lim PE, Zhang H, Gan X, Yong HS. Molecular differentiation and phylogenetic relationships of three *Angiostrongylus* species and *Angiostrongylus cantonensis* geographical isolates based on a 66-kDa protein gene of *A. cantonensis* (Nematoda: Angiostrongylidae). *Exp Parasitol* 2010b; 126: 564-69.

Espírito-Santo MC, Pinto PL, Mota DJ, Gryschek RC**.** The first case of *Angiostrongylus cantonensis* eosinophilic meningitis diagnosed in the city of São Paulo, Brazil. *Rev Inst Med Trop Sao Paulo* 2013; 55: 129-32.

Fontanilla IK, Wade CM. The small subunit (SSU) ribosomal (r) RNA as a genetic marker for identifying infective 3rd juvenile stage *Angiostrongylus cantonensis*. *Acta Trop* 2008; 105: 181-6.

Foronda P, López-González M, Miquel J, *et al*. Finding of *Parastrongylus cantonensis* (Chen, 1935) in *Rattus rattus* in Tenerife, Canary Islands (Spain). *Acta Trop* 2010; 114: 123-7.

Graeff-Teixeira C. Expansion of *Achatina fulica* in Brazil and potential increased risk for angiostrongyliasis. *Trans R Soc Trop Med Hyg* 2007; 101: 743-4.

Harinasuta C, Setasuban P, Radomyos P. Observations on *Angiostrongylus cantonensis* in rats and mollusks in Thailand. *J Med Assoc Thai* 1965; 48: 158-72.

Hongladarom T, Indarakoses A. Eosinophilic meningo-encephalitis caused by *Pila* snail ingestion in Bangkok. *J Med Assoc Thai* 1966: 49: 1-9.

Jefferies R, Shaw SE, Viney ME, Morgan ER. *Angiostrongylus vasorum* from South America and Europe represent distinct lineages. *Parasitology* 2009; 36: 107-15.

Jittayasothorn K, Setasuban P, Keschamrus N. Eosinophilic meningo-encephalitis in Udorn. *Vejasarn Med J* 1965; 14: 1-8.

Khwanmitra S, Bodhidatta A, Punyagupta S. Eosinophilic meningitis. Report of four cases. *J Med Assoc Thai* 1957; 40: 341-3.

Laopornpichayanuwat S. *Angiostrongylus cantonensis* in the cerebrospinal fluid of a female child with eosinophilic meningitis in Thailand. *Siriraj Hosp Gaz* 2000; 52: 553-8.

Lee JD, Chung LY, Wang LC, *et al*. Sequence analysis in partial genes of five isolates of *Angiostrongylus cantonensis* from Taiwan and biological comparison in infectivity and pathogenicity between two strains. *Acta Trop* 2014; 133: 26-34.

Liu C, Zhang R, Chen M, *et al*. Characterisation of *Angiostrongylus cantonensis* isolates from China by sequences of internal transcribed spacers of nuclear ribosomal DNA. *J Anim Vet Adv* 2011; 10: 593-6.

Lv S, Zhang Y, Steinmann P, *et al*. The emergence of angiostrongyliasis in the People's Republic of China: the interplay between invasive snails, climate change and transmission dynamics. *Freshwater Biol* 2011; 56: 717-34.

Maldonado JrA, Simões RO, Oliveira AP, *et al*. First report of *Angiostrongylus cantonensis* (Nematoda: Metastrongylidae) in *Achatina fulica* (Mollusca: Gastropoda) from Southeast and South Brazil. *Mem Inst Oswaldo Cruz* 2010; 105: 938-41.

Monte TCC, Simões RO, Oliveira APM, *et al*. Phylogenetic relationship of the Brazilian isolates of the rat lungworm *Angiostrongylus cantonensis* (Nematoda: Metastrongylidae) employing mitochondrial COI gene sequence data. *Parasit Vectors* 2012; 5: 248-56.

Namue C, Wongsawad C. A survey of helminth infection in rats (*Rattus* spp.) from Chiang Mai moat. *Southeast Asian J Trop Med Public Health* 1997; 28: 179-83.

Nitidandhaprabhas P, Harnsomburana K, Thepsitthar P. *Angiostrongylus cantonensis* in the cerebrospinal fluid of an adult male patient with eosinophilic meningitis in Thailand. *Am J Trop Med Hyg* 1975; 24: 711-12.

Ohbayashi M, Kamiya M, Bhaibulaya M. Studies on parasites fauna of Thailand. I Two new metastrongylid nematodes, *Angiostrongylus siamensis* sp. n. and *Thaistrongylus harinasutai* gen. et. sp. n.

(Metastrongyloidae): Angiostrongylidae fron wild rats. *Jpn J Vet Res* 1979; 27: 5-10.

Panamonta O. Eosinophilic meningitis in children. *Bull Dept Med Services* 1985; 10: 265-9.

Panha S. A checklist and classification of the terrestrial pulmonate snails of Thailand. *Walkerana* 1996; 8: 31-40.

Pipitgool V, Sithithaworn P, Pongmuttasaya P, Hinz E. *Angiostrongylus* infections in rats and snails in northeast Thailand. *Southeast Asian J Trop Med Public Health* 1997; 28: 190-3.

Punyagupta S. Eosinophilic meningoencephalitis in Thailand: summary of nine cases and observations on *Angiostrongylus cantonensis* as a causative agent and *Pila ampullacea* as a new intermediate host. *Am J Trop Med Hyg* 1965; 14: 370-4.

Punyagupta S, Bunnag T, Juttiyudata P, Rosen L. Eosinophilic meningitis in Thailand. Epidemiologic studies of 484 typical cases and the etiologic role of *Angiostrongylus cantonensis*. *Am J Trop Med Hyg* 1970; 19: 950-8.

Sawanyawisuth K, Kithaweesi K, Limpawattana P, *et al*. Intraocular angiostrongyliasis: clinical findings, treatments and outcomes. *Trans R Soc Trop Med Hyg* 2007; 101: 497-501.

Setasuban P, Vairasthira S, Harinasuta C. The preliminary observation on natural infection of rat lung worm (*Angiostrongylus cantonensis*) in rodents and intermediate host in Thailand. *J Med Assoc Thai* 1968; 51: 156-7.

Tamura K, Peterson D, Peterson N, *et al*. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molec Biol Evol* 2011; 28: 2731-9.

Tangchai P, Nye BW, Beaver PC. Eosinophilic meningo-encephalitis caused by angiostrongyliasis in Thailand. Autopsy report. *Am J Trop Med Hyg* 1967; 16: 454-61.

Tantibhedyangur P. A family of meningitis most probably caused by *Angiostrongylus cantonensis*. *J Pediatr Soc Thai* 1963; 2: 77-96.

Teem JL, Qvarnstrom Y, Bishop HS, *et al*. The occurrence of the rat lungworm, *Angiostrongylus cantonensis*, in nonindigenous snails in the Gulf of Mexico region of the United States. *Hawaii J Med Public Health* 2013; 72:11-4.

Tesana S, Srisawangwong T, Sithithaworn P, Laha T, Andrews R. Prevalence and intensity of infection with third stage larvae of *Angiostrongylus cantonensis* in mollusks from Northeast Thailand. *Am J Trop Med Hyg* 2009; 80: 983-7.

Thiengo SC, Maldonado A, Mota EM, *et al*. The giant African snail *Achatina fulica* as natural intermediate host of *Angiostrongylus cantonensis* in Pernambuco, northeast Brazil. *Acta Trop* 2010; 115: 194-9.

Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994; 22: 4673-80.

Tokiwa T, Harunari T, Tanikawa T, *et al*. Phylogenetic relationships of rat lungworm, *Angiostrongylus cantonensis*, isolated from different geographical regions revealed widespread multiple lineages. *Parasitol Int* 2012; 61: 431-6.

Tokiwa T, Hashimoto T, Yabe T, Komatsu N, Akao N, Ohta N. First report of *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae) infections in invasive rodents from five islands of the Ogasawara Archipelago, Japan. *PLOS One* 2013; 7: 8(8): e70729.

Upatham ES, Sornmani S, Kitikoon V, Lohachit C, Burch JB. Identification key for the fresh- and brackish-water snails of Thailand. *Malacol Rev* 1983; 16: 107-32.

Vitta A, Polseela R, Nateeworanart S, Tattiyapong M. Survey of *Angiostrongylus cantonensis* in rats and giant African land snails in Phitsanulok province, Thailand. *Asian Pac J Trop Med* 2011; 4: 597-9.

Vitta A, Polsut W, Fukruksa C, Yimthin T, Thanwisai A, Dekumyoy P. Levels of infection of the lungworm *Angiostrongylus cantonensis* in terrestrial snails from Thailand with *Cryptozona siamensis* as a new intermediate host. *J Helminthol* 2016 Jan 15: 1-5.

Wang QP, Lai DH, Zhu XQ, Chen XG, Lun ZR. Human angiostrongyliasis. *Lancet Infect Dis* 2008; 8: 621-30.

Witoonpanich R, Chuahirun S, Soranastaporn S, Rojanasunan P. Eosinophilic myelomeningoencephalitis caused by *Angiostrongylus cantonensis*: a report of three cases. *Southeast Asian J Trop Med Public Health* 1991; 22: 262-7.

Yang TB, Wu ZD, Lun ZR. The apple snail *Pomacea canaliculata*, a novel vector of the rat lungworm, *Angiostrongylus cantonensis*: its introduction, spread, and control in China. *Hawaii J Med Public Health* 2013; 72: 23-5.

Yong HS, Eamsobhana P, Song SL, Prasartvit A, Lim PE. Molecular phylogeography of *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae) and genetic relationships with congeners using cytochrome b gene marker. *Acta Trop* 2015; 148: 66-71.