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Review Paper

Eosinophilic meningitis caused by *Angiostrongylus cantonensis* – a neglected disease with escalating importance

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Abstract. The rat lungworm *Angiostrongylus cantonensis*, a food-borne zoonotic parasite, has been recognized as the primary pathogen associated with human eosinophilic meningitis or eosinophilic meningoencephalitis. This neurotropic nematode has a definitive rodent host and a molluscan intermediate host. The adult worms live in the pulmonary arteries of rats. Human is a non-permissive, accidental host. Transmission to humans is by eating of infected raw or undercooked snails, poorly cleaned contaminated vegetables or other infected paratenic hosts such as freshwater prawns, crabs, frogs or monitor lizards. Thousands of diagnosed cases of eosinophilic meningitis caused by *A. cantonensis* have been reported worldwide. Angiostrongyliasis is of increasing public health importance as globalization contributes to the geographical spread and more international travelers encounter the disease. The parasite is on the move. It has spread from its traditional endemic areas of Asia and the Pacific Basin to the American continent including the USA, Brazil and Caribbean islands. Recently, the incidence of human infections has increased rapidly. Most reports of the disease are from Thailand and Taiwan with increasing reports from mainland China. The rapid global spread of the parasite and the emerging occurrence of the infection pose challenges in clinical and laboratory diagnosis, and in epidemiology and basic biology. Enhanced understanding of the epidemiology of angiostrongyliasis, increased public awareness about the risks associated with eating raw or undercooked food, and enhanced food safety measures are needed. Therefore, current knowledge on various aspects of the parasite and the disease it causes, as well as recent epidemiological status together with significant progress in laboratory investigation of *A. cantonensis* infection, are overviewed to promote understanding and awareness of this emerging neglected disease.

INTRODUCTION

The rat lungworm *Angiostrongylus cantonensis* was first described in 1933 in the lungs of rats in Canton (now Guangzhou), China, and has now been reported from most parts of the world. It is an emerging zoonotic parasite that has caused thousands of cases of neuro-angiostrongyliasis around the world since the first report of human infection by Nomura and Lin in 1945 in Taiwan (Beaver & Rosen, 1964). *A. cantonensis* is the most important etiological parasite causing eosinophilic meningitis in humans. The nematode is endemic in Asia and the Pacific Basin where most cases of human infection have occurred. High incidence of human infection is observed in Thailand and a number of outbreaks have been documented in mainland China in recent years (Wang *et al.*, 2012; Eamsobhana, 2013). The increase in migrating populations and widespread movement of people due to travel facility has led to the transport of "exotic" foods and pets.

Increased introduction of the parasite hosts (molluscs and rodents) to new settings and climate change have created the opportunity for geographical spread of this parasite (Wang *et al.*, 2012). Today the *A. cantonensis* nematode continues to be reported in new regions beyond its native range. It has spread from its traditional endemic regions to many other regions of the world, including North, Central and South America, Caribbean islands and Africa, where endemic foci for *A. cantonesis* have been discovered and a number of diagnosed cases have been reported (Wang *et al.*, 2008; 2012). Moreover, the parasite has also been recognized as an etiology of eosinophilic meningitis among tourists/travelers returning from the parasite-endemic regions (Wang *et al.*, 2008). The recent establishment of new endemic foci and increasing number of infected cases worldwide highlights the importance of angiostrongyliasis as a risk for human health and has revived interest in this neurotropic parasite.

This mini-review puts together the current knowledge on various aspects of the rat lungworm *A. cantonensis* and the disease it causes. Updated epidemiological status together with significant progress in laboratory investigation of *A. cantonensis* infection are highlighted to draw attention of angiostrongyliasis to the medical community (e.g. clinicians, clinical laboratory personnel and health officers) and to raise general public awareness of this emerging infectious disease. Moreover, an international collaborative network to develop a concerted and rigorous research agenda to address angiostrongyliasis (rat lungworm disease) at a global scale through updating/advancing an integrated understanding of all aspects of the parasite and disease, in particular on the evaluation of diagnostic methods that provide opportunity for multi-center blind testing, and to share/distribute tasks to avoid repetitive efforts to improve the pace for developing well standardized diagnostic systems for human infection with *A. cantonensis* are discussed.

Life Cycle of *Angiostrongylus cantonensis*

The life cycle of *A. cantonensis* is well documented (Bhaibulaya, 1975). It involves rats as definitive hosts and snails or slugs as intermediate hosts, and also paratenic hosts such as freshwater shrimps, crabs, frogs, toads and monitor lizards, that feed on molluscs (Eamsobhana & Tungtrongchitr, 2005). Human is an accidental host, acquiring the infection by eating raw or undercooked infected intermediate snail host, or other infected paratenic hosts, or by eating raw vegetables contaminated by a small infected snail or slug.

Adult male (20 to 25 mm by 0.32 to 0.42 mm) and female (22 to 34 mm by 0.34 to 0.56 mm) (Figure 1) live in the pulmonary arteries of rats. The females lay eggs and hatch in the terminal branches of the pulmonary arteries. First-stage larvae migrate to the pharynx, are then swallowed and passed in the feces. The larvae penetrate or are ingested by an intermediate snail host. The third-stage larvae are infective to rodents/humans. When the mollusc is ingested by the definitive host, the third-stage larvae migrate to the brain where they develop into young adults. The young adults return to the venous system and then the pulmonary arteries where they become sexually mature. In accidental human host, infective larvae migrate to the brain and develop into juvenile worms, where

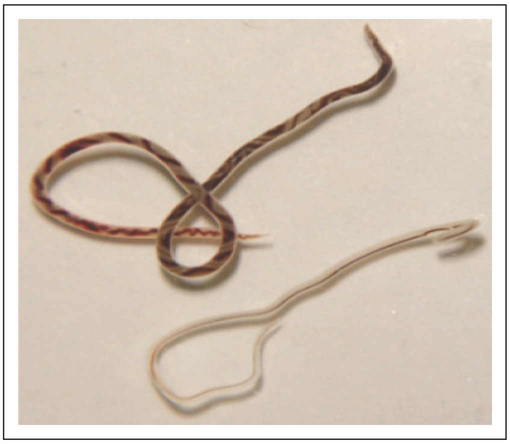

Figure 1. *Angiostrongylus cantonensis* adult female (upper) and male (lower) worms.

Figure 2. Typical Thai 'koi-hoi' dish.

they ultimately die, leading to intense inflammatory responses and various neurological lesions/disorders and even death.

Angiostrongyliasis and Eosinophilic Meningitis

Man is a non-permissive, accidental host of *A. cantonensis*. Human angiostrongyliasis occurs sporadically or in outbreaks in endemic countries. Previously, cases of eosinophilic meningitis caused by *A. cantonensis* have been reported from Southeast Asia and the Pacific islands. Presently, the infection is spreading to many other regions outside its traditional geographical boundaries, including Americas, Africa, Australia and the Caribbean (Wang *et al.*, 2008; 2012).

In Thailand, the disease was first recognized in 1955, with four documented cases in 1957 (Khwanmitra *et al.*, 1957). The number of cases increased rapidly in the 1960s (Punyagupta *et al.*, 1970). Of the 1,164 cases reported from 1955 to 1966, 912 were from the northeastern provinces where a typical snail dish 'koi-hoi' is popular (Punyagupta *et al.*, 1970). The dish is usually prepared from chopped raw snail meat, usually the *Pila* spp., and flavored with seasoning and vegetables, e.g. minced roasted-rice, onion, lime or lemon juice, chili, fish sauce, parsley and mint leaves (Figure 2). The dish is consumed immediately after preparation along with a local alcoholic drink. Moreover, a fatal case with infection attributed to eating raw or partially cooked livers from monitor lizards was recorded at Siriraj Hospital, Bangkok in 1990 (Eamsobhana & Tungtrongchitr, 2005). A very high infection rate of 95.5% (21/22 lizards) with the infective larvae of *A. cantonensis* was identified from the yellow tree monitor *Varanus nebulosus* in Thailand (Radomyos *et al.*, 1994). By 2008, at least 1,345 diagnosed cases with angiostrogyliasis have been recorded in Thailand (Wang *et al.*, 2012). Furthermore, ocular angiostrongyliasis, a rare manifestation, has also been documented occasionally in Thailand (Sawanyawisuth *et al.*, 2007). Of the 654 cases diagnosed with angiostrongyliasis at Srinagarin Hospital in Khon Kaen during 1995-2005, 7 (1.1%) were with ocular manifestations (Sawanyawisuth *et al.*, 2007).

Although the first report of human case was described from Taiwan in the 1960s, there has been no case recorded until 1984, whence several outbreaks were detected in mainland China. In recent years, cases of human angiostrongyliasis increased significantly in P.R. China due to the increase of living standards and income, and the modern food consumption trends. By the end of 2009, angiostrongyliasis cases/outbreaks have been reported from at least 9 provinces in P.R. China, where 457 cases had been identified (Wang *et al.*, 2012). The five cities, Wenzhou, Fuzhou, Guangzhou, Kunming and Beijing accounted for 88.9% of the cases (Lv *et al.*, 2008; Lv, 2011). At least 75.3% of all cases were attributed to the raw consumption of two snail species, *Pomacea canaliculata* and *Achatina fulica* (Lv *et al.*, 2008; Lv, 2011).

Over the past decade, eosinophilic meningitis cases due to *A. cantonensis* have been increasingly recognized in Hawaii. Although the parasite was first recognized in Hawaii in the early 1960s, human cases were not reported until late 2004 when a case cluster was seen on the Island of Hawaii, and the State made angiostrongyliasis a reportable disease in 2005 (Kwon, *et al.*, 2013; Park & Fox, 2013; Wallace, 2013). From 2005 through 2011, the Hawaii State Department of Health had received reports of 38 cases of autochthonous angiostrongyliasis eosinophilic meningitis (Kwon, *et al.*, 2013; Park & Fox, 2013; Wallace, 2013). While Hawaii appears to be the primary state where endemic cases have been documented, uncommon/rare cases of eosinophilic meningitis caused by *A. cantonensis* have been found in other states in the Continental United States (Miller *et al.*, 2014).

A. cantonensis infection was previously restricted to the Pacific Islands and most Asian countries, but human infections was first reported in Brazil in 2007 with 3 documented cases from the southeastern state of Espirito Santo, followed by an additional case in Pernambuco in the northeast region in 2009 (Simões *et al.*, 2011). Since then, intermediate snail hosts (e.g. *Achatina fulica*) and definitive rodent hosts (Norway rats) infected with *A. cantonensis* have been identified in many states in the northern, northeastern, southeastern, and southern regions of Brazil (Simões *et al.*, 2011; Espirito-Santo, 2013; Moreira, 2013).

Over the past decade, sporadic imported cases with *A. cantonensis* infection have been reported in travelers after returning from Pacific islands and Caribbean islands (Wang, 2008; 2012). Given the increasingly widespread travel of the populace, there is a trend that more and more travelers/tourists from non-endemic countries returning from *A. cantonensis* endemic areas are being diagnosed with neuro-angiostrongyliasis. In travel medicine, the disease has now been considered to be one of the possible neurological diseases that need differential diagnosis.

Clinical Presentation

Infection with *A. cantonensis* is typically associated with peripheral eosinophilia, and a high eosinophil count in the cerebrospinal fluid (CSF), accounting for at least 10% of the total CSF leukocyte count. Incubation period of cerebral angiostrongyliasis varies from one day to several months, depending on the number of ingested larvae. The common clinical presentations are eosinophilic meningitis and ocular angiostrongyliasis. Cases with fatal encephalitic angiostrongyliasis have also been reported (Sawanyawisuth *et al.*, 2009; Wang *et al.*, 2012).

Clinical symptoms are caused by the presence of larvae or developing worms and local host reactions in the brain. Dead and dying worms in the central nervous system can provoke more inflammatory reactions and disease (Cross & Chen, 2007). The main symptoms with eosinophilic meningitis are severe headache, neck stiffness, paresthesia, vomiting and nausea. The recent combined data from Thailand, Taiwan, mainland China, and the United States of America showed that adult patients infected with *A. cantonensis* had headache (95%), mild stiffness of the neck (46%), persistent paresthesia (44%), vomiting (38%) and nausea (28%) (Wang *et al.*, 2012). In children, cases with neck stiffness and paresthesia were less common, whereas nausea and vomiting were more common. In adults and children with heavy load of parasites, symptoms often progress to unconsciousness, coma and even death (Wang *et al.*, 2012).

Investigations

Any suspected case of *A. cantonensis* infection can only be confirmed by finding the worms in CSF or eye chamber of the patients (a rare occurrence). Presumptive diagnosis is based on specific eating habits, symptoms of severe headache, meningitis/meningoencephalitis and ocular involvement, CSF and peripheral blood eosinophilia. A history of eating intermediate/paratenic hosts (raw or undercooked) is crucial for diagnosis. The various clinical presentations must be differentiated from those caused by cerebral gnathostomiasis, neurocysticercosis, and cerebral paragonimiasis. Brain imaging results (MRI, CT) to detect damage in brain can be useful for differential diagnosis (Jaroonvesama, 1988). Immunological tests using enzyme-linked immunosorbent assay (ELISA) method to detect specific antibodies against *A. cantonensis* are useful and help in differential diagnosis (Eamsobhana & Yong, 2009).

Angiostrongyliasis, as a neglected disease, does not attract sufficient resources from commercial test developers because costs are not rewarded with justifiable profits. As such, many laboratories develop their own tests. Several specific *A. cantonensis* antigens have been identified and used in different laboratories to detect specific *A. cantonensis* antibodies. Immunoblot test to detect specific antibody has been reported to distinguish infections with *A. cantonensis*, *Gnathostoma spinigerum*, and cysticerci of *Taenia solium*. The 31-kDa band identified by immuno-blotting is a diagnostic marker for human angiostrongyliasis. The sensitivity and specificity of the test were both 100% (Eamsobhana, 1994). At Siriraj Hospital, Bangkok, the 31-kDa glycoprotein antigen of *A. cantonensis* has been used as diagnostic marker for diagnosis of human angiostrongyliasis (Figure 3). A cheap and simple dot blot-ELISA has been developed and proven to be convenient for field use (Eamsobhana *et al.*, 2004). Positive result is indicated by a colored spot on nitrocellulose strip. Multi-dot ELISA to distinguish eosinophilic meningitis due to clinically related parasitic infections (angiostrongyliasis, gnathostomiasis and neurocysticercosis) has been developed (Figure 4) (Eamsobhana *et al.*, 2006). Various parasite antigens (*A. cantonensis*, *G. spinigerum* and *T. solium* cysticerci) were dotted on a membrane strip and reacted against a given patient's serum.

Recently, a more rapid non-enzymatic, filtration-based dot immunogold assay (DIGFA) using purified 31-kDa antigen (Figure 5) to detect specific antibody against *A. cantonensis* in infected patients has been developed. DIGFA has performed well on clinical samples at Siriraj Hospital (Eamsobhana *et al.*, 2014). It is now being validated on serum samples collected from *A. cantonensis* endemic areas in Thailand. Although enzyme-coupled ELISA methods are well accepted as immunologic tests for diagnosis, technical difficulties with the procedure and time constraint limit their use in most laboratories/field studies. The 3-min DIGFA test will likely replace the 3-hr dot-

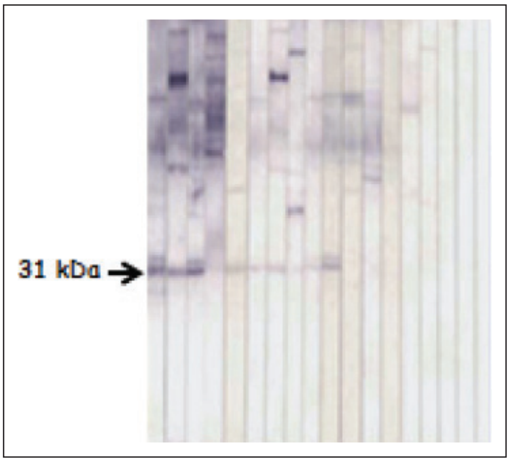

Figure 3. Immunoblot analysis of clinical serum samples. Arrow indicates the diagnostic 31-kDa band for *A. cantonensis* infection.

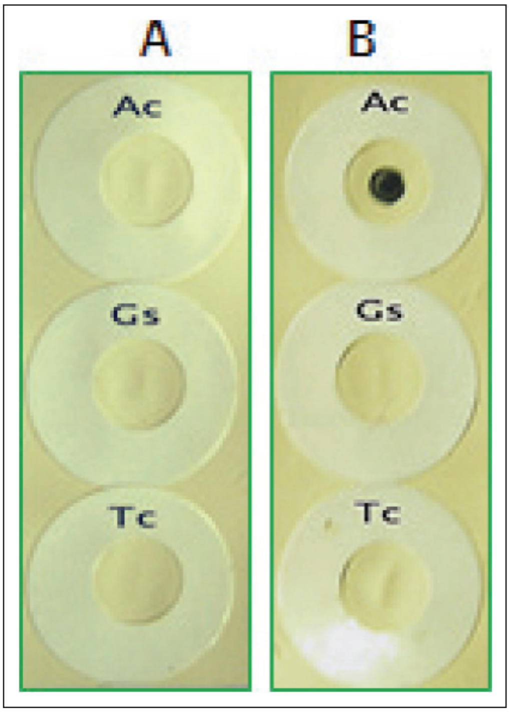

Figure 4. Multi-dot ELISA using purified antigens of *Angiostrongylus cantonensis* (Ac), *Gnathostoma spinigerum* (Gs), and *Taenia solium* cysticerci (Tc). A colored dot on strip B shows positive reaction only for angiostrongyliasis.

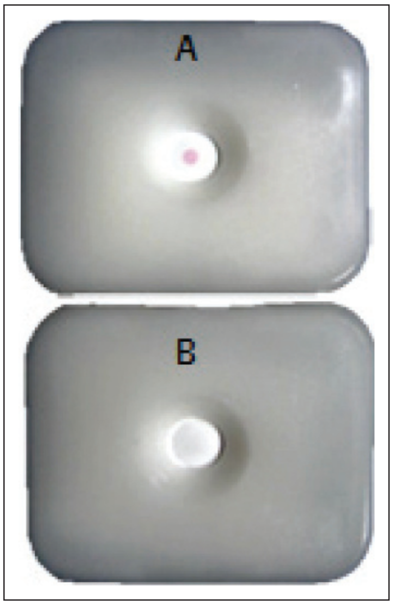

Figure 5. DIGFA with positive (A) and negative (B) results.

ELISA/immunoblot for both clinical use and screening of eosinophilic meningitis patients in areas where *A. cantonensis* is endemic. DIGFA is promising as a future diagnostic test kit. Improvements of DIGFA to also differentiate other clinically related parasites in a multi-dot test format would broaden its applicability.

The characterization of several genes of *A. cantonensis* in recent years has paved the way for the use of molecular techniques for diagnosis of angiostrongyliasis. PCR using primers (AC1, AC2) based on mRNA sequence encoding a 66-kDa protein gene of *A. cantonensis* amplified a fragment of 300 bp in CSF from 4 out of 10 sero-positive patients (Eamsobhana *et al.*, 2013). More CSF samples from confirmed angiostrongyliasis patients are needed. More specific primers are still required, and more development, evaluation, dissemination of tests and validation appear necessary.

Treatment

Most human cases with eosinophilic meningitis caused by *A. cantonensis* are usually mild and symptoms can resolve spontaneously without any treatment. Severe cases that lead to long-term neurological complications are occasional. In severe cases, treatment relies on symptomatic relief. Supportive treatment includes the use of corticosteroids, adequate analgesia and therapeutic CSF aspiration to relieve increasing intracranial pressure (Cross, 1987). There is no effective curative treatment for angiostrongyliasis (Cross, 1987). Avoidance of anthelminthic agents has been recommended due to the potential harm from the inflammatory response provoked by the dead worms (Cross, 1987).

Steroid treatment appears to be beneficial. The 2-week course of oral corticosteroid (60 mg/day) is the recommended treatment for meningitis caused by *A. cantonensis* (Chotmongkol *et al.*, 2006; 2009). The combination therapy of corticosteroid and anthelminthic (albendazole or mebendazole) has been reported, but the efficacy is similar to corticosteroid alone (Chotmongkol *et al.*, 2006; 2009; Sawanyawisuth & Sawanyawisuth, 2008). There is no effective treatment for encephalitic and ocular angiostrongyliasis. In patients with ocular manifestation, surgery is required to remove worms from the eyes, but visual outcome depends on initial visual defects (Sawanyawisuth & Sawanyawisuth, 2008; Sawanyawisuth *et al.*, 2007).

Prevention and Control of Angiostrongyliasis

Control of *A. cantonensis* parasite in nature is nearly impossible but community control measures could be implemented. In any area where *A. cantonensis* has never been reported, if suspected cases of eosinophilic meningitis or meningoencephalitis show up, research should be initiated to investigate if the parasite has already reached there. The identification of the source of human infection will allow local health authorities to implement public information campaigns about the risk of eating raw or undercooked meat, which render the infection with *A. cantonensis* in the region. Clinicians should also contact local public health services for updated information on sources for diagnostic tests.

Food safety is a legitimate concern, especially for many food-borne parasitic infections, including angiostrongyliasis. Human infection with *A. cantonensis* is of increasing concern in food safety, which has led to adjustments in food processing protocols to ensure quality in food production everywhere. Nevertheless, changes in eating habits that have been practiced for generations can be difficult. Populations in the parasite endemic areas should be made aware of the dangers of eating raw or undercooked snails and paratenic animal hosts.

The populations should also be informed of hazards of accidentally eating slugs and terrestrial planarians that are often found in gardens and on vegetables (Wang *et al.*, 2008; 2012). Attention to personal hygiene, hand-washing after outdoor work, washing vegetables thoroughly, avoid eating of raw or undercooked molluscs or crustaceans, and provision of clean drinking water could contribute to the prevention of infection with the rat lung worm, *A. cantonensis* (Hollyer, 2013).

Angiostrongyliasis as a Neglected Disease

Human angiostrongyliasis has attracted increasing public attention worldwide due to many outbreaks, and because more and more sporadic cases are being reported among international travelers in recent years. To date, at least 2,877 human cases of *A. cantonensis*-induced eosinophilic meningitis had been documented in approximately 30 countries (Wang *et al.*, 2012). However, many more cases are unreported or unrecognised due to lack of awareness of this parasite within the medical community and the general public. The infection is re-emerging due to changes in dietary habits of people, increased global transportation of food produce as well as increased international trade and travelling.

The increasing number of cases in Thailand and worldwide during the past decade called for a more thorough study of the parasite, disease and epidemiology. An international symposium on "*Angiostrongylus* and angiostrongyliasis" was then initiated in Bangkok, 8-9 April 2010, with its theme "advances in the disease, control, diagnosis, treatment and molecular genetics of the genus *Angiostrongylus*". The workshop was well attended by 29 participants (clinicians and scientists) from 7 countries. A cooperative network has been set up in order to share current data and to establish future projects leading to prevention and control and better treatment. The proceedings of this symposium, representing work done in the endemic regions and countries where this parasite/disease is present or has emerged, had been published and distributed to provoke awareness in the medical community (Eamsobhana, 2011).

Due to the emergence and increased frequency of occurrence of eosinophilic meningitis cases in Hawaii, the 2nd Rat Lungworm Workshop was held in Honolulu from 16-18 August 2011, expanding on the scope of the previous one in Bangkok. The workshop covered ecology, parasitology, epidemiology, detection, diagnosis, treatment as well as food safety. Research and outreach were identified and a prioritized list of objectives was developed and the top needs in eight areas were highlighted, i.e., detection of parasite in hosts, control of hosts in the field (rats, slugs/snails, paratenic hosts), public education to minimize chance of infection, control of hosts/larvae on produce (e.g., washing/rinsing), diagnosis, treatment, pathophysiology, and epidemiology. A conference summary in the Journal of Emerging Infectious Diseases and a special issue of the Hawaii Journal of Medicine and Public Health (included 24 articles) are part of the effort to raise awareness of the parasite/disease (Cowie *et al.*, 2012; Cowie, 2013). This was followed by the 3rd Rat Lungworm Workshop held 22-25 November 2013, at Sun Yat-Sen University, where the parasite was first described in rat by Professor Chen Xin-Toa, in Canton (Guangzhou), P.R. China, to further update on the parasite and disease and to facilitate/enhance more research collaboration. The conference summary of the workshop is underway.

As the life cycle of *A. cantonensis* in rat was first elucidated at the University of

Queensland in Brisbane (Mackerras & Sandars, 1955; Bhaibulaya, 1971), the next 4th Rat Lungworm Workshop has been proposed to be hosted by the University of Queensland/QIMR Berghofer Medical Research Institute, Brisbane, Australia in 2016. Presently, an active search for useful panels of well-defined and purified antigens, preferably recombinant molecules, is urgently needed. The urgent need is also for standardization of molecular detection methods to detect *A. cantonensis* nucleic acids that can be applied to clinical CSF samples. There is an ongoing effort to establish a world-wide multicenter laboratory network for evaluation and quality control of diagnostic methods for angiostrongyliasis. It is hoped that, more updated data will be shared and more collaborative research projects will be established, leading to better diagnosis and treatment as well as effective prevention and control of *A. cantonensis* infection in humans. Furthermore, multi-center validation studies in order to standardize methods for detection/diagnosis of this parasite/disease can be fully implemented.

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REFERENCES

Beaver, P.C. & Rosen, L. (1964). Memorandum on the first report of *Angiostrongylus* in man, by Nomura and Lin, 1945. *American Journal of Tropical Medicine and Hygiene* **13**: 589-590.

Bhaibulaya, M. (1971). *Angiostrongylus mackerrasae* and *Angiostrongylus cantonensis*: studies on speciation, life history and host-parasite relationship. PhD Thesis. Australia: University of Queensland.

Bhaibulaya, M. (1975). Comparative studies of the life history of *Angiostrongylus mackerrasae* (Bhaibulaya, 1968) and *Angiostrongylus cantonensis* (Chen, 1935). *International Journal of Parasitology* **5**: 7-20.

Chotmongkol, V., Sawadpanitch, K., Sawanyawisuth, K., Louhawilai, S. & Limpawattana, P. (2006). Treatment of eosinophilic meningitis with a combination of prednisolone and mebendazole. *American Journal of Tropical Medicine and Hygiene* **74**: 1122-1124.

Chotmongkol, V., Kittimongkolma, S., Niwattayakul, K., Intapan, P.M. & Thavornpitak, Y. (2009). Comparison of prednisolone plus albendazole with prednisolone alone for treatment of patients with eosinophilic meningitis. *American Journal of Tropical Medicine and Hygiene* **81**: 443-445.

Cross, J.H. (1987). Public health importance of *Angiostrongylus cantonensis* and its relatives. *Parasitology Today* **3**: 367-369.

Cross, J.H. & Chen, E.R. (2007). Angiostrongyliasis. In: *Food borne parasitic zoonoses* (Editors, K.D. Murrel & B. Fried) pp. 263-290. Springer, New York.

Cowie, R.H., Hollyer, J.R., da Silva, A.J., Hollingsworth, R.G., C. Dixon, M.C., Eamsobhana, P., Fox, L.M., Gosnell, W.L., Howe, K., Johnson, S., Kim, J.R., Kramer, K.J., Lim, P.E., Lindo, J.F., Lun, Z.R., Maldonado, A., Morassutti, A.L., Murphy, G.S., Park, S.Y., Qvarnstrom, Y., Robinson, R.D., Sawanyawisuth, K., Teem, J., Thiengo, S.C., Todd, C.D., Tsai, H.C., Wallace, G.D., Waugh, C.A., Whelen, A.C., Wilkins, P.P., Yang, T.B. & Yong, H.S. (2012). Workshop on research priorities for management and treatment of angiostrongyliasis. *Emerging Infectious Diseases* **18**(12): e1.doi: 10.3201/eid1812.120499.

Cowie, R.H. (2013). Eosinophilic meningitis caused by *Angiostrongylus cantonensis*, the rat lungworm: biology, distribution, epidemiology, detection, diagnosis, treatment, and management. *Hawaii Journal of Medicine and Public Health* **72**: 28-32.

Eamsobhana, P. (1994). Immunological studies on the rat lung-worm *Angiostrongylus cantonensis* (Nematoda: Metastrongylidae). PhD Thesis. Kuala Lumpur: University of Malaya.

Eamsobhana, P. (2011). *Angiostrongylus* and Angiostrongyliasis: *advances in the disease*, *control*, *diagnosis and molecular genetics*. Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok.

Eamsobhana, P. (2013). Angiostrongyliasis in Thailand: epidemiology and laboratory investigations. *Hawaii Journal of Medicine and Public Health* **72**: 28-32.

Eamsobhana, P. & Tungtrongchitr, A. (2005). Angiostrongyliasis in Thailand. In: *Food-borne helminthiasis in Asia*. (Editors, N. Arizono, J.Y. Chai, Y. Nawa & T. Takahashi) pp.183-197. Chiba, The Federation of Asian Parasitologists, Japan.

Eamsobhana, P. & Yong, H.S. (2009). Immunological diagnosis of human angiostrongyliasis cantonensis. *International Journal of Infectious Diseases* **13**: 425-431.

Eamsobhana, P., Yoolek, A., Punthuprapasa, P. & Suvouttho, S. (2004). A dot-blot ELISA comparable to immunoblot for the specific diagnosis of human parastrongyliasis. *Journal of Helminthology* **78**: 287-291.

Eamsobhana, P., Ongrotchanakun, J., Yoolek, A., Monkong N., Punthuprapasa, P. & Dekumyoy, P. (2006). Multi-immunodot for rapid differential diagnosis of eosinophilic meningitis due to parasitic infections. *Journal of Helminthology* **80**: 1-7.

Eamsobhana, P., Wanachiwanawin, D., Dechkum, N., Parsartvit, A. & Yong, H.S. (2013). Molecular diagnosis of eosinophilic meningitis due to *Angiostrongylus cantonensis* (Nematoda: Metastrongyloidea) by PCR-DNA sequencing of cerebrospinal fluids of patients. *Memorias do Instituto Oswaldo Cruz* **108**: 116-118.

Eamsobhana, P., Gan, X.X., Wanachiwanawin, D., Ma, A., Wang, Y. & Yong, H.S. (2014). Dot immunogold filtration assay (DIGFA) for the rapid detection of specific antibodies against the rat lungworm *Angiostrongylus cantonensis* (Nematoda: Metastrongyloidea) using purified 31-kDa antigen. *Journal of Helminthology* **88**: 396-401.

Espirito-Santo, M.C., Pinto, P.L., da Mota, D.J. & Gryschek, R.C. (2013). The first case of *Angiostrongylus cantonensis* eosinophilic meningitis diagnosed in the city of Sao Paulo, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo* **55**:129-32.

Holler, J.R. (2013). Telling consumers, gardeners, and farmers about the possible risk of rat lungworm in the local food supply in Hawaii. *Hawaii Journal of Medicine and Public Health* **72**: 82.

Jaroonvesama, N. (1988). Differential diagnosis of eosinophilic meningitis. *Parasitology Today* **4**: 262-266.

Khwanmitra, S., Bodhidatta, A. & Punyagupta, S. (1957). Eosinophilic meningitis. Report of four cases. *Journal of Medical Association of Thailand* **40**: 341-343.

Kwon, E., Ferguson, T.M., Park, S.Y., Manuzak, A., Qvarnstrom, A.Y., Morgan, S., Ciminera, P. & Murphy, G.S. (2013). A severe case of *Angiostrongylus* eosinophilic meningitis with encephalitis and neurologic sequelae in Hawaii. *Hawaii Journal of Medicine and Public Health* **72**: 41-45.

Lv, S. (2011). Epidemiology of *Angiostrongylus cantonensis* and eosinophilic meningitis in the People's Republic of China. PhD Thesis. Basel: University of Basel.

Lv, S., Zhang, Y., Steinmann, P. & Zhou, X.N. (2008). Emerging angiostrongyliasis in mainland China. *Emerging Infectious Diseases* **14**: 161-164.

Mackerras, M.J. & Sandars, D.F. (1955). The life history of the rat lungworm, *Angiostrongylus cantonensis* (Chen) (Nematoda: Metastrongylidae). *Australian Journal of Zoology* **3**: 1-25.

Miller, M.A, Menowsky, M., Leeson, K. & Leeson, B. (2014). Eosinophilic meningitis: what's the 'diff'? *American Journal of Emergency Medicine* **32**: 107.e5-107.e7.

Moreira, V.L.C., Giese, E.G., Melo, F.T.V., Simoes, R.O., Thiengo, S.C., Maldonado, J.Y.A. & Santo, S.J.N. (2013). Endemic angiostrongyliasis in the Brazilian Amazon: natural parasitism of *Angiostrongylus cantonensis* in *Rattus rattus* and *R. norvergicus*, and sympatric giant African land snails, *Achatina fulica*. *Acta Tropica* **125**: 90-97.

Park, S.Y. & Fox, L.M. (2013). *Angiostrongylus cantonensis*: epidemiology in the Continental United States and Hawaii. *Hawaii Journal of Medicine and Public Heath* **72**: 34.

Punyagupta, S., Bunnag, T., Juttiyudata, P. & Rosen, L. (1970). Eosinophilic meningitis in Thailand. Epidemiologic studies of 484 typical cases and the etiologic role of *Angiostrongylus cantonensis*. *American Journal of Tropical Medicine and Hygiene* **19**: 950-958.

Radomyos, P., Tungtrongchitr, A. & Praewanich, R. (1994). Occurrence of the infective stage of *Angiostrongylus cantonensis* in the yellow tree monitor (*Varanus bengalensis*) in five provinces of Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **25**: 498-500.

Sawanyawisuth, K. & Sawanyawisuth, K. (2008). Treatment of angiostrongyliasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102**: 990-996.

Sawanyawisuth, K., Kithaweesi, K., Limpawattana, P., Intapan, P.M., Tiamkao, S., Jitpimolmard, S. & Chotmongkol, V. (2007). Intraocular angiostrongyliasis: clinical findings, treatments and outcomes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **101**: 497-501.

Sawanyawisuth, K., Takahashi, K., Hoshuyama, T., Senthong, V., Limpawattana, P., Intapan, P.M., Wilson, D., Tiamkao, S., Jitpimolmard, S.,Chotmongkol, V. (2009). Clinical factors predictive of encephalitis caused by *Angiostrongylus cantonensis*. *American Journal of Tropical Medicine and Hygiene* **81**: 698-701.

Simoes, R., Monteiro, F., Sánchez, E., Thiengo, S.C., Garcia, J.S., Costa-Neto, S.F., Luque, J.L. &. Maldonado, A. (2011). Endemic angiostrongyliasis, Rio de Janeiro, Brazil. *Emerging Infectious Diseases* **17**: 1331-1333.

Wallace, G.D. (2013). The discovery of humans in Hawaii infected with *Angiostrongylus cantonensis*, and early epidemiological findings. *Hawaii Journal of Medicine and Public Health* **72**: 5.

Wang, Q.P., Lai, D.H., Zhu, X.Q., Chen, X.G. & Lun, Z.R. (2008). Human angiostrongyliasis. *Lancet Infectious Disease* **8**: 621-630.

Wang, Q.P., Wu, Z.D., Wei, J., Owen, R.L. & Lun, Z.R. (2012). Human *Angiostrongylus cantonensis*: an update. *European Journal of Clinical Microbiology and Infectious Diseases* **31**: 389-395.