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Angiostrongylus cantonensis: a review of its distribution, molecular biology and clinical significance as a human pathogen

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SUMMARY

Angiostrongylus cantonensis is a metastrongyloid nematode found widely in the Asia-Pacific region, and the aetiological agent of angiostrongyliasis; a disease characterized by eosinophilic meningitis. *Rattus* rats are definitive hosts of *A. cantonensis*, while intermediate hosts include terrestrial and aquatic molluscs. Humans are dead-end hosts that usually become infected upon ingestion of infected molluscs. A presumptive diagnosis is often made based on clinical features, a history of mollusc consumption, eosinophilic pleocytosis in cerebral spinal fluid, and advanced imaging such as computed tomography. Serological tests are available for angiostrongyliasis, though many tests are still under development. While there is no treatment consensus, therapy often includes a combination of anthelmintics and corticosteroids. Angiostrongyliasis is relatively rare, but is often associated with morbidity and sometimes mortality. Recent reports suggest the parasites' range is increasing, leading to fatalities in regions previously considered *Angiostrongylus*-free, and sometimes, delayed diagnosis in newly invaded regions. Increased awareness of angiostrongyliasis would facilitate rapid diagnosis and improved clinical outcomes. This paper summarizes knowledge on the parasites' life cycle, clinical aspects and epidemiology. The molecular biology of *Angiostrongylus* spp. is also discussed. Attention is paid to the significance of angiostrongyliasis in Australia, given the recent severe cases reported from the Sydney region.

Key words: Angiostrongyliasis, *Angiostrongylus*, Diagnosis, Eosinophilic meningitis, Molluscs, Rat, Lungworm, Clinical significance, Epidemiology, Molecular biology.

INTRODUCTION

Angiostrongylus cantonensis (common name: the rat lungworm) is a metastrongyloid nematode originally identified in the Guangzhou region of China in the brown rat, *Rattus norvegicus* (Chen, 1935). It was first assigned to the genus *Pulmonema* which was later synonymized with *Angiostrongylus* (Dougherty, 1946) resulting in the current combination, *A. cantonensis*. It was identified as a human pathogen in 1945 (Beaver and Rosen, 1964) and is now recognized as the leading cause of eosinophilic meningitis worldwide (Wang *et al*. 2008; Graeff-Teixeira *et al*. 2009; Murphy and Johnson, 2013). It was the aetiological agent in over 2877 human cases of eosinophilic meningitis and is gaining recognition as an emerging zoonosis (Wang *et al*. 2012). Dogs and certain wildlife species are important biosentinels for angiostrongyliasis, highlighting the potential risk for humans living in regions where these species are affected (Ma *et al*. 2013).

While *A. cantonensis* is the leading cause of eosinophilic meningitis, several other aetiological agents must be considered (Graeff-Teixeira *et al*. 2009). Serological tests for angiostrongyliasis are commercially available, though have not been widely adopted for routine use. Consequently, diagnosis is often intuitive, relying on an accurate patient history and non-specific tests such as computed tomography (CT) or magnetic resonance imaging (MRI). A patients travel history or history of mollusc consumption greatly assist the diagnostic endeavour. Microscopic examination of cerebrospinal fluid (CSF) can be helpful, though the low sensitivity of this technique limits its use (Eamsobhana and Yong, 2009). Given the potential lethality of angiostrongyliasis, it must be given due consideration in all cases of eosinophilic meningitis.

Traditionally, *A. cantonensis* is endemic to the temperate and tropical parts of the Far East (York *et al*. 2014), though its current range includes

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Southeast Asia, the Pacific Islands, parts of South and Central America and the Caribbean (Wang *et al*. 2008). Relatively new epidemiological data have emerged from Australia and the USA, suggesting that the parasite's geographical range is expanding (Teem *et al*. 2013; Chan *et al*. 2015; Iwanowicz *et al*. 2015). Australian cases of angiostrongyliasis have been reported since the 1970s, possibly as early as 1959 (Prociv and Carlisle, 2001), though interest in this parasite was renewed due to recent reports of lethal cases on Australia's eastern coast.

A review summarizing current knowledge on various aspects of *A. cantonensis* is provided. This includes its life cycle and transmission, the global epidemiology of angiostrongyliasis, clinical manifestations, current diagnostic approaches, therapy, and measures for control and prevention. The molecular biology of *A. cantonensis* is also discussed. Particular attention is paid to the significance of angiostrongyliasis in Australia given the increasing reports of angiostrongyliasis in Australian humans and wildlife (Blair *et al*. 2013; Ma *et al*. 2013; Morton *et al*. 2013; Spratt, 2015). An increased awareness of this emerging zoonosis is paramount to improving the prognosis for affected patients and reducing human, and companion animal suffering as the range of the parasite increases.

CLASSIFICATION

Angiostrongylus cantonensis is placed in the family Angiostrongylidae in the superfamily Metastrongyloidea which includes over 180 species across 45 genera. *Angiostrongylus cantonensis* was originally described as *Pulmonema cantonensis* by Chen (1935) and was subsequently placed in the genus *Angiostrongylus* by Dougherty (1946). Later, Ubelaker (1986) split *Angiostrongylus* into five genera: *Angiostrongylus* (in carnivores), *Parastrongylus* (murids), *Angiocaulus* (mustelids), *Gallegostrongylus* (gerbils and one murid) and *Sterfanskostrongylus* (insectivores), though this classification, placing *A. cantonensis* in *Parastrongylus*, is now rarely used (Cowie, 2013*a*).

Twenty-one species of *Angiostrongylus* are currently recognized, with the most significant being *A. costaricensis* and *A. cantonensis*, given their role as zoonotic pathogens (Spratt, 2015). Other significant species include *Angiostrongylus malaysiensis*, *Angiostrongylus vasorum* and *Angiostrongylus mackerrasae*. These species are considered animal pathogens but their zoonotic potential is yet to be demonstrated (Spratt, 2015).

LIFE CYCLE AND TRANSMISSION

Elucidation of the *A. cantonensis* life cycle was initially accredited to Mackerras and Sandars (1955) who studied what they believed to be *A. cantonensis* in *R. norvegicus* captured on river banks in Queensland, Australia. These parasites were later identified as *A. mackerrasae*, although the life cycles of *A. cantonensis*, *A. mackerrasae* and *A. malaysiensis* are extremely similar, differing mostly in their host preferences (Bhaibulaya, 1975; Spratt, 2015). *Angiostrongylus costaricensis* also has a similar life cycle, though possesses a tropism for the mesenteric arteries of its definitive host, where egg production takes place (Mota and Lenzi, 2005). In contrast, *A. cantonensis* produces eggs in the pulmonary arteries of its definitive hosts (Thiengo *et al*. 2013; Spratt, 2015). The life cycle of *A. cantonensis* (Fig. 1) involves one of many potential intermediate hosts (molluscs) and definitive hosts (various rat species), and a large number of potential paratenic hosts.

Rattus rattus (the black rat) and *R. norvegicus* (the brown rat) are the favoured definitive hosts of *A. cantonensis*, though at least 17 rodent species may behave as definitive hosts, capable of passing first-stage (L_1) larvae (Fig. 2) in their feces (Yong and Eamsobhana, 2013). Simoes *et al*. (2014) postulated that the movement of *A. cantonensis* to new regions is mediated mostly by male rats that often have greater dispersal ability than females, while females are important for maintenance of *A. cantonensis* on a small, local scale. Rats become infected by ingestion of an intermediate or a paratenic host, containing third-stage (L_3) *A. cantonensis* larvae (Fig. 2). A few hours after ingestion, L_3 larvae penetrate the rats' intestinal wall and enter its blood stream where they are dispersed via the circulation. Larvae that reach the brain undergo an additional moult to become fourth-stage (L_4) larvae. A fifth moult ensues in the subarachnoid space, where larvae enter the young adult (L_5) stage. Young adult worms leave the central nervous system (CNS) and migrate through the circulation, and at 25 dpi, are found in the pulmonary arteries. At 35 dpi, adults have reached sexual maturity and females begin producing eggs that hatch in the terminal branches of the pulmonary arteries, liberating L_1 larvae. These larvae penetrate the alveolae, migrate to the pharynx and are swallowed by the rat. The L_1 larvae then travel through the digestive tract and appear in the rats' feces approximately 42 days after the initial exposure (Thiengo *et al*. 2013).

Definitive host competency may vary between rat species. For example, the Australian indigenous rat, *Rattus fuscipes*, seems a poor definitive host for *A. cantonensis* but is thought to be the preferred host of *A. mackerrasae* (Prociv and Carlisle, 2001). Similarly, the prevalence of *A. cantonensis* is usually much higher in *R. norvegicus* compared with *Rattus flavipectus* (Zhang *et al*. 2008*a*; Deng *et al*. 2012). This could be attributable to the biological competencies of different rat species, or simply, due to differences in the dietary habits of

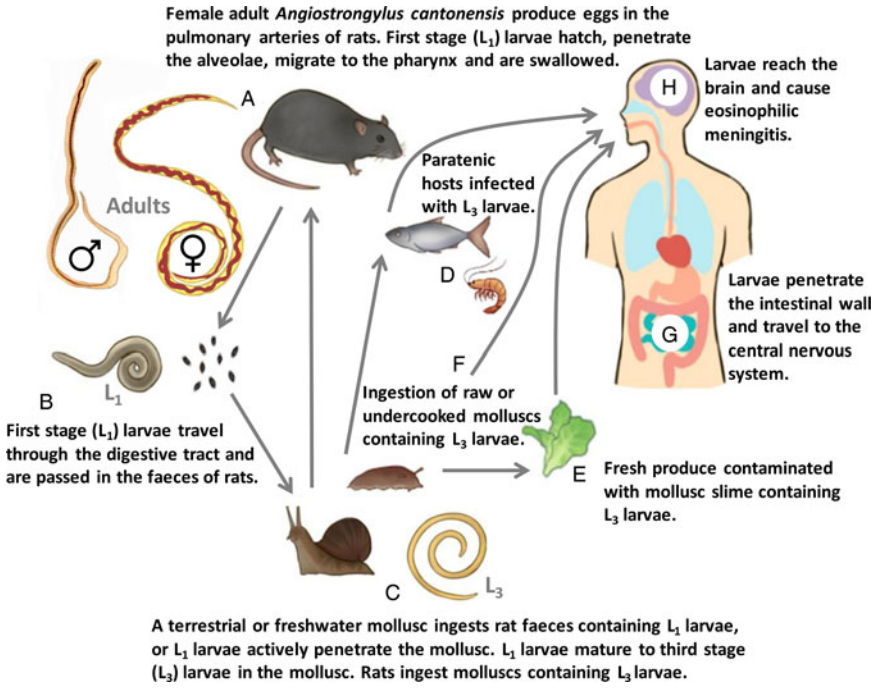

Fig. 1. Life cycle of *A. cantonensis*. (A) Male (♂) and female (♀) adult *A. cantonensis* live in the pulmonary arteries of *Rattus* rats, their preferred definitive host. Females lay eggs that hatch in the terminal branches of the pulmonary arteries, liberating first-stage (L_1) larvae. The L_1 larvae penetrate the alveolae, migrate to the pharynx and are swallowed. (B) The L_1 larvae travel through the digestive tract and are passed in the rat feces. (C) A terrestrial or freshwater mollusc ingests the rat feces containing L_1 larvae, or L_1 larvae actively penetrate the mollusc tegument. The L_1 larvae undergo two moults in the mollusc to become third-stage (L_3) larvae. Infected molluscs are then ingested by a rat. The L_3 larvae penetrate the rats' intestine and migrate via the circulation to the brain where they undergo two additional moults to become young adult (L_5) worms. The L_5 worms leave the CNS and travel through the circulation to the pulmonary arteries where they mature to adulthood and reproduce. (D) Paratenic hosts eat molluscs infected with L_3 larvae, and the larvae become quiescent in these hosts. Infected paratenic hosts remain infectious to accidental hosts such as humans. (E) Fresh produce contaminated with mollusc slime may also represent a source of human infection, though direct ingestion of raw or undercooked molluscs (F) is the most common route of human infection. (G) Once ingested, L_3 larvae penetrate the intestinal wall and travel through the blood stream to the central nervous system. (H) The larvae enter the brain and in accidental hosts such as humans, eventually die. A granulomatous inflammatory reaction in the CNS is caused in response to dead worms, which manifests as eosinophilic meningitis.

different rat species, though this is yet to be ascertained (Zhang *et al*. 2008*a*). Non-*Rattus* rats such as the bandicoot rat (*Bandicota indica*) and the white-toothed rat (*Berylmys berdmorei*), are also definitive hosts of *A. cantonensis* (Pipitgool *et al*. 1997; Deng *et al*. 2012; Yong and Eamsobhana, 2013). The finding of adult *A. cantonensis* in the Ryukyu Islands tree rat (*Diplothrix legata*) (Okano *et al*. 2014) also implicates this species as a definitive host, though this requires further investigation. Experimental infections in Mongolian gerbils (*Meriones unguiculatus*) confirmed they are highly susceptible to angiostrongyliasis yet behave as poor definitive hosts; worms developed to sexual maturity in gerbils though very few L_1 larvae were shed in their feces (Wei *et al*. 2014).

Molluscs become infected by ingesting rat feces or by active penetration of L_1 larvae through their tegument (Morassutti *et al*. 2014). First-stage larvae remain viable for several days after being excreted, though viability drops sharply after this (Yousif and Lammler, 1975*a*). The efficiency of

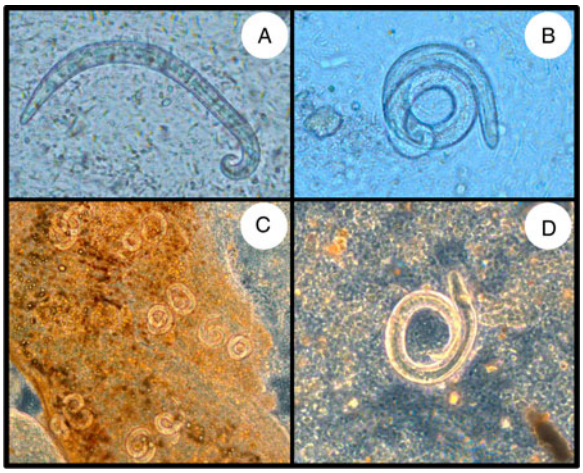

Fig. 2. Microscopic observation of L_1 (A, B) and L_3 (C, D) *A. cantonensis* larvae from rat feces and mollusc tissue, respectively. Each L_1 larvae is approximately 320 μM in length, while L_3 larvae are slightly larger, at approximately 400 μM in length. The L_1 larvae were imaged under bright-field microscopy, while the L_3 larvae were imaged under phase-contrast microscopy.

mollusc infection is temperature dependent; it is greater at 26 °C compared with 24 °C (Yousif and Lammler, 1975*a*). Within the mollusc, L_1 larvae undergo two moults to become L_3 larvae; a roughly 20-day process marked by distinct morphological changes (Lv *et al*. 2009*c*; Thiengo *et al*. 2013; Zeng *et al*. 2013*b*). Larvae often migrate to the muscular layer of the foot given its excellent vascular supply, which provides favourable conditions for metastrongyloid larvae development (Mendonca *et al*. 1999; Giannelli *et al*. 2015). Larvae also migrate to the lung tissues (Yousif *et al*. 1980; Lv *et al*. 2009*c*). Consequently, mollusc lungs are often examined microscopically in field studies, for the characteristic nodules that contain L_3 larvae (Lv *et al*. 2009*c*; Hu *et al*. 2011; Qvarnstrom *et al*. 2013). In the aquatic snail *Pila polita*, ingested larvae migrate from the digestive gland to the intestine and then to the mantle, where the highest parasite burdens were recorded (Tesana *et al*. 2008). In *Parmarion martensi* semi-slugs (semi-slugs are molluscs with a shell that is too small to retract into), the midsection and tail had the highest larval densities; few larvae were found in the head (Jarvi *et al*. 2012). *Angiostrongylus cantonensis* is also pathogenic to molluscs, inducing biochemical disturbances, inflammation and reduction in reproductive capacity (Yousif *et al*. 1980; Tunholi-Alves *et al*. 2011, 2012, 2014, 2015).

Intermediate hosts of *A. cantonensis* include species from as many as 51 mollusc families, though some species may harbour more L_3 larvae than others (Table 1) (Yousif and Lammler, 1975*b*; Kim *et al*. 2014). When the aquatic snails *Pomacea canaliculata* and *P. polita* were experimentally infected with *A. cantonensis*, higher larval burdens were observed in *P. polita* (Tesana *et al*. 2008). The giant African land snail, *Achatina fulica*, may harbour thousands of L_3 larvae, and is thought to be a major contributor to the spread of *A. cantonensis* globally (Alicata, 1965*a*), though this is controversial (Civeyrel and Simberloff, 1996; Cowie, 2013*a*). The effect of mollusc size on larval burden has also been considered, though correlations were only observed in some species (Yousif and Lammler, 1975*a*; Pipitgool *et al*. 1997; Ibrahim, 2007; Tesana *et al*. 2009). It is suggested that parasite burdens are more closely linked to the number of L_1 larvae that the molluscs have been exposed to (Yousif and Lammler, 1975*a*). The prevalence of *A. cantonensis* in mollusc populations also varies between locations (Table 2), probably due to environmental factors such as temperature, humidity, the distribution of molluscs and the behaviours of local rats (Kim *et al*. 2014). Water salinity is also a contributing factor, with higher prevalences observed in aquatic molluscs living in water with lower salinity (Ibrahim, 2007).

In China and parts of Southeast Asia, *P. canaliculata*, and in Thailand, *Pila* spp., are considered important intermediate hosts of *A. cantonensis* (Lv *et al*. 2009*b*; Tesana *et al*. 2009; Eamsobhana, 2013; Yang *et al*. 2013*a*). The number of known intermediate host species is increasing, with recent reports describing infections in new species from mollusc families such as the Achatinellidae, Assimineidae, Oxychilidae, and the species *Theba pisana, Plutonia lamarckii, Zachrysia provisoria, Cornu aspersum* and *Cryptozona siamensis* (Table 2) (Kim *et al*. 2014; Vitta *et al*. 2016). In Australia, two terrestrial snail species, *C. aspersum* and *Bradybaena similaris* (Fig. 3) were recently confirmed as natural intermediate hosts of *A. cantonensis* (Table 2). Third-stage larvae of *A. cantonensis* also infect a multitude of paratenic hosts including freshwater prawns & shrimp, land crabs, frogs, toads, monitor lizards and planarians (Cowie, 2013*b*; Qvarnstrom *et al*. 2013; Eamsobhana, 2014). The L_3 larvae remain infective to definitive and accidental hosts that eat infected paratenic hosts.

Humans are one of many accidental hosts that become infected via one of the three possible transmission pathways (Fig. 1D–F). In Australia, naturally acquired infections in other accidental hosts have involved dogs, horses, brushtail possums (Fig. 4), Bennett's wallabies, rufous bettongs, tamarins (in captivity), black and grey-headed flying foxes, tawny frogmouths (Fig. 4, online Supplementary materials), gang–gang cockatoos and yellow-tailed black cockatoos (McKenzie *et al*. 1978; Wright *et al*. 1991; Higgins *et al*. 1997; Carlisle *et al*. 1998; Barrett *et al*. 2002; Monks *et al*. 2005; Lunn *et al*. 2012; Reece *et al*. 2013; Aghazadeh *et al*. 2015*a*; Walker *et al*. 2015). It has been suggested that brushtail possums and tawny frogmouths are susceptible dead-end hosts native to Australia that serve as biosentinels for the presence of *A. cantonensis* (Ma *et al*. 2013; Spratt, 2015). In the USA, infections in several dead-end hosts have also been confirmed, including a privately owned orangutan with a history of snail consumption (Emerson *et al*. 2013), and a captive gibbon from a zoo in Miami (Duffy *et al*. 2004). Burns *et al*. (2014) described a fatal case in a captive African pygmy falcon from San Diego Zoo, Southern California, USA. Kottwitz *et al*. (2014) reported cases of lethal angiostrongyliasis in captive Geoffroy's tamarins from a zoo in Alabama, USA.

Deliberate ingestion of infected raw or undercooked molluscs is the most common route of infection for humans in Asian countries, such as Thailand, where known intermediate hosts, such as *Pila* spp., are regularly eaten raw as part of the local diet (Eamsobhana, 2013). Human *A. cantonensis* infections have also been linked to several paratenic host species. Reports describing the ingestion of monitor lizards in Sri Lanka, Thailand & India, raw frogs in China and, freshwater shrimp, fish & crabs in the Pacific Islands, implicate ingestion of these disparate hosts as a route for acquiring

Table 1. Larval parasite loads (infection intensities) in naturally infected intermediate and paratenic hosts of *A. cantonensis*

Mollusc species	Location	Number of larvae identified per infected mollusc	Reference
Terrestrial snails			
Achatina fulica	Kamphaeng Phet Province, Thailand	Min = 5, Max = 15	Vitta *et al.* (2016)
	Brazil	Mean = 220·5–833·6, Max = 3108	Oliveira *et al.* (2015)[a]
	Hawaii Island, Hawaii, USA	Mean = 5, Median = 1, Max = 18	Qvarnstrom *et al.* (2013)[b]
	Oahu, Hawaii, USA	Mean = 7, Median = 3, Max = 25	Qvarnstrom *et al.* (2013)[b]
	Tahiti, French Polynesia	Min = 11, Mean = 18·2, Median = 18, Max = 28	Fontanilla and Wade (2012)
	Panyu region, Guangzhou City, China	Mean = 57	Chen *et al.* (2012*a*)
	Northeast Thailand	Min = 5, Mean = 849·8, Median = 140·5, Max = 3113	Tesana *et al.* (2009)
	Northeast Thailand	Mean ≅ 6	Pipitgool *et al.* (1997)[c]
Bradybaena similaris	Sydney, Australia	Max = 0·01–1	Chan *et al.* (2015)[d]
Cornu aspersum	Brisbane, Australia	Max = 2	Aghazadeh *et al.* (2015*b*)
	Sydney, Australia	Max = 0·01 to 1	Chan *et al.* (2015)[d]
Cryptozona siamensis	Kamphaeng Phet Province, Thailand	Min = 1, Max = 4858	Vitta *et al.* (2016)
Aquatic snails			
Biomphalaria alexandrina	North Sinai, Egypt	Mean = 1·5–3	Ibrahim (2007)[e]
Cleopatra cyclostomoides	North Sinai, Egypt	Mean = 1·75–7	Ibrahim (2007)[e]
Cleopatra bulimoides	North Sinai, Egypt	Mean = 1–3·36	Ibrahim (2007)[e]
Lanistes carinatus	North Sinai, Egypt	Mean = 2–37·62	Ibrahim (2007)[e]
Lymnaea natalensis	North Sinai, Egypt	Mean = 2	Ibrahim (2007)
Melanoides tuberculata	North Sinai, Egypt	Mean = 1·33–3	Ibrahim (2007)[e]
Pila pesmei	Northeast Thailand	Min = 1, Mean = 20·2, Median = 3, Max = 159	Tesana *et al.* (2009)
Pomacea canaliculata	Panyu region, Guangzhou City, China	Mean = 1·64	
	Southern Taiwan	Mean values: A = 60, B = 49, C = 65, D = 36, E = 53	Wang *et al.* (2011)[f]
	Guangdong Province, China	6000 in one snail, >1000 in two others	Li *et al.* (2008)
Pomacea maculata	Louisiana, USA	Mean = 19, Median = 2, Max = 71	Qvarnstrom *et al.* (2013)[b]
Semi-slugs			
Parmarion martensi	Hawaii Island, Hawaii, USA	Mean = 445, Median = 110, Max ≧ 1000	Qvarnstrom *et al.* (2013)[b]
	Hawaii, USA	Mean = 0·10–60	Jarvi *et al.* (2012)[g]
Terrestrial slugs			
Laevicaulis alte	Hawaii Island, Hawaii, USA	Mean = 205, Median = 2, Max = 819	Qvarnstrom *et al.* (2013)[b]
Pallifera spp.	Hawaii Island, Hawaii, USA	Mean ≦ 1, Median ≦ 1, Max ≦ 1	Qvarnstrom *et al.* (2013)[b]
Veronicella cubensis	Hawaii Island, Hawaii, USA	Mean = 35, Median ≦ 1, Max = 468	Qvarnstrom *et al.* (2013)[b]
Other			
Other mollusc species	Hawaii Island, Hawaii, USA	Mean ≦1, Median ≦ 1, Max ≦ 1	Qvarnstrom *et al.* (2013)[b]
	Northeast Thailand	Min = 1, Mean = 6·7, Median = 6, Max = 13	Tesana *et al.* (2009)
Planarians (paratenic hosts)	Hawaii Island, Hawaii, USA	Mean = 4, Median ≦ 1, Max = 30	Qvarnstrom *et al.* (2013)[b]

[a] Mean infection intensity varied between months.
[b] Mean infection intensity is per 25 mg of tissue.
[c] The mean infection intensity for L_3 larvae from all worm species was 13·6. Infection of Wistar rats with these larvae resulted in a recovery rate of 48·3% adult worms and 91·7% of these (~ 6) were *A. cantonensis*.
[d] Mean infection intensity is per 50 mg of tissue.
[e] Mean infection intensity varied between seasons and/or locations.
[f] Snails from irrigation canals in five different locations (designated A–E).
[g] Mean infection intensity is per 1 mg of tissue. Also, the mean infection intensity varied depending on the mollusc tissue sample screened (mollusc head, mid-section, tail or slime), and the location from which molluscs were collected.

Table 2. Prevalence of *A. cantonensis* in definitive, intermediate and paratenic hosts

Host species	Location	Prevalence	Reference
Definitive hosts			
Bandicota indica	Guangdong Province, China	1/6 (16·7%)	Deng *et al*. (2012)
	Northeast Thailand	1/69 (1·5%)	Pipitgool *et al*. (1997)
Rattus exulans	Hawaii Island, Hawaii, USA	10/10 (100%)	Qvarnstrom *et al*. (2013)[a]
Rattus flavipectus	Shenzhen, Guangdong Province, China	6/121 (4·9%)	Zhang *et al*. (2008*a*)
	Guangdong Province, China	3/118 (2·5%)	Deng *et al*. (2012)
Rattus fuscipes	Brisbane, Queensland, Australia	14/53 (26%)	Aghazadeh *et al*. (2015*b*)
Rattus lutreolus	Brisbane, Queensland, Australia	2/5 (40%)	Aghazadeh *et al*. (2015*b*)
Rattus norvegicus	Brisbane, Queensland, Australia	4/15 (27%)	Aghazadeh *et al*. (2015*b*)
	New Orleans, Louisiana, USA	2/36 (5·6%)	York *et al*. (2015)
	Trindade, São Gonçalo, Brazil	78/110 (70·9%)	Simoes *et al*. (2014)
	Hawaii Island, Hawaii, USA	1/1 (100%)	Qvarnstrom *et al*. (2013)
	Islands in the Ogasawara Archipelago, Japan	46 /57 (80·7%)	Tokiwa *et al*. (2013)
	Guangdong Province, China	52/263 (19·8%)	Deng *et al*. (2012)
	Guangzhou, Guangdong Province, China	20/220 (9·1%)	Yang *et al*. (2012)
	Hainan, China	13/118 (11·0%)	Hu *et al*. (2011)
	Guangdong Province, China	310/1835 (16·9%)	Pan *et al*. (2011)
	São Gonçalo, Rio de Janeiro, Brazil	19/27 (70·4%)	Simoes *et al*. (2011)
	Grenada, West Indies	45/192 (23·4%)	Chikweto *et al*. (2009)
	Shenzhen, Guangdong Province, China	31/187 (16·6%)	Zhang *et al*. (2008*a*)
	Jamaica	20/78 (25·6%)	Lindo *et al*. (2002)
	Northeast Thailand	2/52 (3·8%)	Pipitgool *et al*. (1997)
	Northeast Puerto Rico	19/40 (47·5%)	Andersen *et al*. (1986)
Rattus rattus	Brisbane, Queensland, Australia	52/325 (16%)	Aghazadeh *et al*. (2015*b*)
	Hawaii Island, Hawaii, USA	26/26 (100%)	Qvarnstrom *et al*. (2013)[a]
	Tenerife, Canary Islands	30/54 (55·6%)	Martin-Alonso *et al*. (2011)[b]
	Phitsanulok Province, Thailand	1/62 (1·6%)	Vitta *et al*. (2011)
	Tenerife, Canary Islands	10/67 (15%)	Foronda *et al*. (2010)
	Jamaica	4/31 (12·9%)	Lindo *et al*. (2002)
	Northeast Puerto Rico	10/63 (15·9%)	Andersen *et al*. (1986)
Sigmodon hispidus[c]	McCurtain County, Oklahoma, USA	1/30 (3·3%)	York *et al*. (2015)
Intermediate hosts			
Achatina fulica	Kamphaeng Phet Province, Thailand	3/275 (1·1%)	Vitta *et al*. (2016)
	Miami, Florida, USA	18/50 (36%)	Iwanowicz *et al*. (2015)
	São Gonçalo, Rio de Janeiro, Brazil	78·7% of 153	Oliveira *et al*. (2015)
	China	21·5% of 21 138	Song *et al*. (2016)[d]
	Hawaii Island, Hawaii, USA	7/8 (87·5%)	Qvarnstrom *et al*. (2013)
	Oahu, Hawaii, USA	5/9 (55·6%)	Qvarnstrom *et al*. (2013)
	Miami, Florida	4/140 (2·9%)	Teem *et al*. (2013)
	Panyu region, Guangzhou, China	83/367 (22·6%)	Chen *et al*. (2012*a*)
	Guangdong Province, China	223/1354 (16·5%)	Deng *et al*. (2012)
	Qushui Village, Suixi County, Zhanjiang City	13%	Shen *et al*. (2012)
	Guangzhou, China	111/795 (14%)	Yang *et al*. (2012)
	Hainan, China	121/534 (22·7%)	Hu *et al*. (2011)
	Hawaii, USA	5/6 (83·3%)	Qvarnstrom *et al*. (2010)[e]
	Pernambuco, Northeast Brazil	14/33 (42·4%)	Thiengo *et al*. (2010)
	China[f]	13·4% of 3549	Lv *et al*. (2009*b*)
	Northeast Thailand	4/53 (7·6%)	Tesana *et al*. (2009)
	Shenzhen, Guangdong Province, China	31/302 (10·3%)	Zhang *et al*. (2008*a*)
	Jiangmen, Guangdong Province, China	45% of 695	Zhang *et al*. (2008*b*)
	Santa Catarina Island, Florianopolis, Brazil	1/244 (0·4%)	Neuhauss *et al*. (2007)
Achatina spp.	Phitsanulok Province, Thailand	38/307 (12·4%)	Vitta *et al*. (2011)
Alcadia striata	Southern Florida, USA	1/19 (5·3%)	Stockdale-Walden *et al*. (2015)[g]
Ampularum crossean	Suixi County, Zhanjiang, China	10%	Shen *et al*. (2012)
Bellamya aeruginosa	Fuzhou and Xiamen, Fujian Province, China	31/19 843 (0·2%)	Li *et al*. (2013*a*)
Bellamya spp.	Guangdong Province, China	1/70 (1·4%)	Deng *et al*. (2012)
	Guangdong Province, China	3/91 (3·3%)	Deng *et al*. (2012)
Biomphalaria alexandrina	North Sinai, Egypt	3/294 (1%)	Ibrahim (2007)
Bradybaena similaris	Sydney, Australia	1/10 (10%)	Chan *et al*. (2015)
	São Gonçalo, Rio de Janeiro, Brazil	24·6% of 245	Oliveira *et al*. (2015)
	Southern Florida, USA	3/7 (42·9%)	Stockdale-Walden *et al*. (2015)[g]
Cepaea spp.	Hainan, China	9/43 (20·9%)	Hu *et al*. (2011)
Cleopatra bulimoides	North Sinai, Egypt	41/3588 (1·1%)	Ibrahim (2007)
Cleopatra cyclostomoides	North Sinai, Egypt	45/3401 (1·3%)	Ibrahim (2007)
Cornu aspersum	Brisbane, Queensland, Australia	1/87 (1·2%)	Aghazadeh *et al*. (2015*b*)[h]
	Sydney, Australia	14/312 (4·5%)	Chan *et al*. (2015)
	Tenerife, Canary Islands, Spain	9/18 (50%)	Martin-Alonso *et al*. (2015)[i]
Cryptozona siamensis	Kamphaeng Phet Province, Thailand	45/425 (10·6%)	Vitta *et al*. (2016)
Hemiplecta distincta	Northeast Thailand	2/10 (20%)	Tesana *et al*. (2009)

Table 2. (*Cont.*)

Host species	Location	Prevalence	Reference
Laevicaulis alte	Hawaii Island, Hawaii, USA	4/5 (80%)	Qvarnstrom *et al.* (2013)
	Hawaii, USA	4/5 (80%)	Qvarnstrom *et al.* (2010)[e]
Lanistes carinatus	North Sinai, Egypt	56/2490 (2·2%)	Ibrahim (2007)
Lymnaea natalensis	North Sinai, Egypt	2/218 (0·9%)	Ibrahim (2007)
Melanoides tuberculata	North Sinai, Egypt	26/4112 (0·6%)	Ibrahim (2007)
Pallifera spp.	Hawaii Island, Hawaii, USA	2/5 (40%)	Qvarnstrom *et al.* (2013)
Parmarion martensi	Hawaii Island, Hawaii, USA	77/97 (79·4%)	Qvarnstrom *et al.* (2013)
	Hawaii, USA	83/112 (74·1%)	Qvarnstrom *et al.* (2010)[e]
	Hawaii, USA	25/34 (73·5%)	Qvarnstrom *et al.* (2007)
Phlegm bilineatus	Hainan, China	146/517 (28·2%)	Hu *et al.* (2011)
Pila pesmei	Northeast Thailand	11/454 (2·4%)	Tesana *et al.* (2009)
Pila polita	Northeast Thailand	9/181 (5%)	Tesana *et al.* (2009)
	Northeast Thailand	4/423 (0·9%)	Pipitgool *et al.* (1997)
Plutonia lamarckii	Tenerife, Canary Islands, Spain	43/60 (71·7%)	Martin-Alonso *et al.* (2015)[i]
Pomacea canaliculata	China	7·6% of 41 299	Song *et al.* (2016)[d]
	Fuzhou and Xiamen, Fujian Province, China	753/5744 (13·8%)	Li *et al.* (2013*a*)
	Panyu region, Guangzhou City, China	3·08% of 357	Chen *et al.* (2012*a*)
	Guangdong Province, China	172/2929 (5·87%)	Deng *et al.* (2012)
	Guangzhou, China	11/734 (1·5%)	Yang *et al.* (2012)
	Hainan, China	64/518 (12·4%)	Hu *et al.* (2011)
	Southern Taiwan	109/535 (20·4%)	Wang *et al.* (2011)
	China[f]	6·8% of 11 709	Lv *et al.* (2009*b*)
	Shenzhen, Guangdong Province, China	65/314 (20·7%)	Zhang *et al.* (2008*a*)
	Jiangmen, Guangdong Province, China	1·8% of 720	Zhang *et al.* (2008*b*)
Pomacea insularum	Louisiana, USA	5/31 (16·1%)	Qvarnstrom *et al.* (2010)[e]
Pomacea lineata	Pernambuco, Northeastern Brazil	5/95 (5·3%)	Thiengo *et al.* (2010)
Pomacea maculata	New Orleans, Louisiana, USA	5/31 (16·1%)	Qvarnstrom *et al.* (2013)
	Gretna, Louisiana, USA	8/60 (13·3%)	Teem *et al.* (2013)
	Mandeville, Louisiana, USA	1/40 (2·5%)	Teem *et al.* (2013)
Theba pisana	Tenerife, Canary Islands, Spain	7/20 (35%)	Martin-Alonso *et al.* (2015)[i]
Thelidomus asper	Mandeville, Jamaica	4/10 (40%)	Lindo *et al.* (2002)
Veronicella cubensis	Hawaii Island, Hawaii, USA	27/71 (38%)	Qvarnstrom *et al.* (2013)
	Hawaii, USA	23/50 (46%)	Qvarnstrom *et al.* (2010)[e]
Zachrysia provisoria	Southern Florida, USA	7/34 (20·6%)	Stockdale-Walden *et al.* (2015)[g]
Other aquatic snails	China[f]	0·05% of 7287	Lv *et al.* (2009*b*)
	Northeast Thailand	4/841 (0·5%)	Tesana *et al.* (2009)
Other terrestrial snails	China[f]	0·3% of 1421	Lv *et al.* (2009*b*)
Other terrestrial slugs	China[f]	6·5% of 5370	Lv *et al.* (2009*b*)
Other molluscs	Hawaii Island, Hawaii, USA	6/14 (42·9%)	Qvarnstrom *et al.* (2013)
	Hawaii, USA	5/16 (31·3%)	Qvarnstrom *et al.* (2010)[e]
Paratenic hosts			
Planarians	Hawaii Island, Hawaii, USA	8/12 (66·7%)	Qvarnstrom *et al.* (2013)
	Hawaii, USA	2/2 (100%)	Qvarnstrom *et al.* (2010)[e]

[a] This study compared the necropsy of rats with real-time PCR, for confirmation of an *A. cantonensis* infection. This table shows the higher prevalence value.
[b] This is a study on the seroprevalence of *A. cantonensis* in rats and indicates exposure to worms rather than an active, current infection.
[c] *Sigmodon hispidus* is an important definitive host of *A. costaricensis* (Graeff-Teixeira *et al.* 1990), though it has not yet been described as a definitive host of *A. cantonensis* (Yong and Eamsobhana, 2013).
[d] A meta-analysis involving 38 studies from different regions in China, over a 10-year period.
[e] This study compared conventional and real-time PCR for detection of *A. cantonensis* in mollusc tissues. This table shows the higher prevalence value.
[f] This study involved 164 counties from 19 provinces, predominantly along southeastern coastline of China.
[g] This study compared microscopic identification of worms from pepsin digested mollusc tissue to PCR, for detection of *A. cantonensis* in mollusc tissues. This table shows the higher prevalence value.
[h] The prevalence shown for this study was generated from a group of molluscs containing mixed species, in which one *C. aspersum* was infected only.
[i] This study compared microscopic identification of worms from pepsin digested mollusc tissue to a loop-mediated isothermal amplification assay, for detection of *A. cantonensis*. This table shows the higher prevalence value.

Angiostrongylus infections (Hidelaratchi *et al.* 2005; Malvy *et al.* 2008; Tsai *et al.* 2011; Cowie, 2013*b*; Pai *et al.* 2013; Eamsobhana, 2014).

The shedding of *A. cantonensis* larvae in infected mollusc mucus onto fresh produce may represent another route of transmission (Fig. 1E). This pathway is relevant to those that regularly eat uncooked, inadequately washed, plant material, i.e. in salads (Barrow *et al.* 1996; Lindo *et al.* 2002; Slom *et al.* 2002; Waugh *et al.* 2005; Yeung *et al.* 2013).

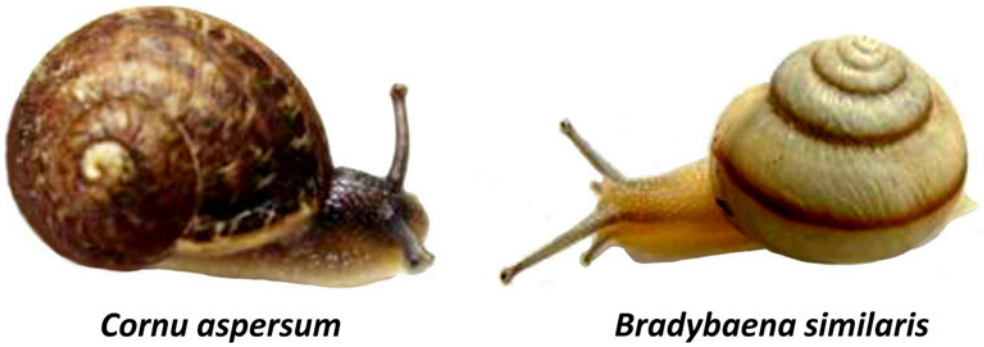

Fig. 3. Two terrestrial mollusc species that were identified as natural intermediate hosts of *A. cantonensis* in Australia. *Cornu aspersum* and *Bradybaena similaris* are introduced species in Australia, and are common inhabitants of gardens and parks in metropolitan areas along the eastern coast of the continent.

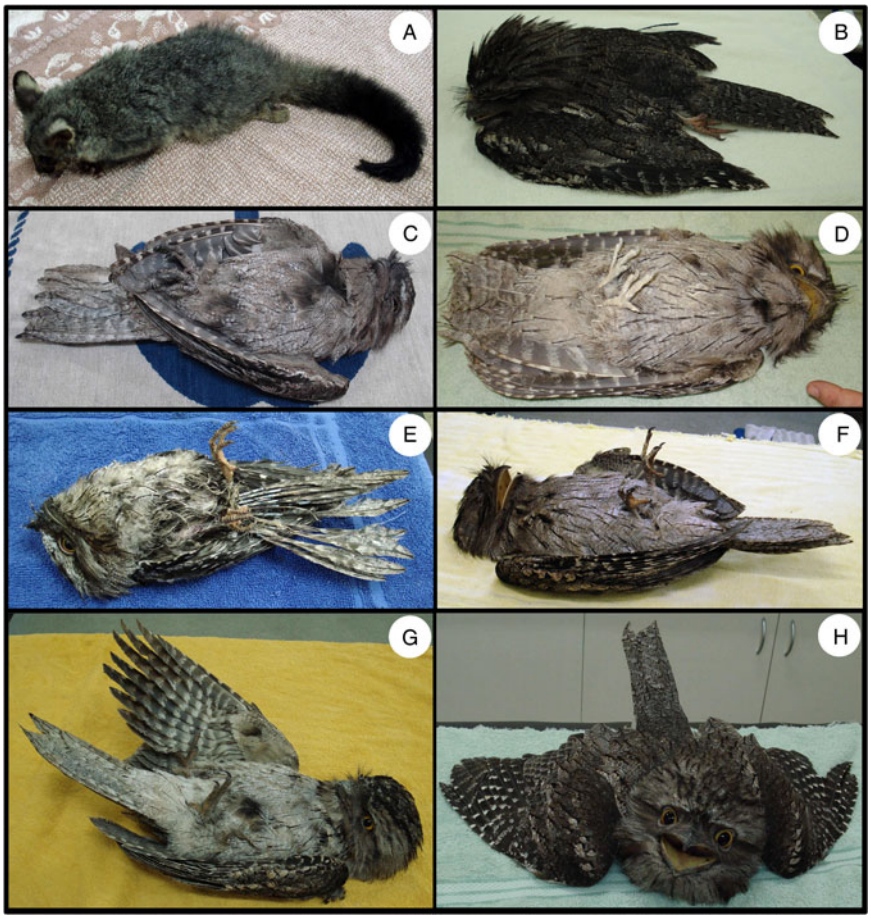

Fig. 4. Clinical presentation of angiostrongyliasis in some Australian wildlife species, including a brushtail possum (*Trichosurus vulpecula*) (A) and multiple tawny frogmouths (*Podargus strigoides*) (B–H). These animals were described as biosentinels for *A. cantonensis* in the Sydney region (Ma *et al.* 2013). Panel (A) shows a juvenile brushtail possum that had been in care for 6 weeks. Food supplied to its large cage was gradually being left uneaten, but food offered to the possum in its nest box was eaten ravenously. Closer examination showed an inability to ambulate. Clinical examination showed hind limb and tail paralysis. Pinching the hind paws elicited no pain response but a strong and even exaggerated withdrawal reflex, typical for spinal cord damage. Panels (B–H) show tawny frogmouths displaying signs typical for spinal cord damage, prior to a diagnosis of angiostrongyliasis. The birds present with varied clinical signs, from moribund in advanced cases (B), to reduced or normal mentation (C–H). Some birds may be alert and aware but unable to fly or stand (H).

An outbreak has also been linked to drinking raw vegetable juices (Tsai *et al.* 2004, 2013). Independent studies that included several mollusc species, reported *A. cantonensis* in mollusc mucus, though the small number of larvae shed may be negligible or insufficient to represent a major source of human infection (Qvarnstrom *et al.* 2007; Jarvi *et al.* 2012; Chan *et al.* 2015). However, mollusc slime was

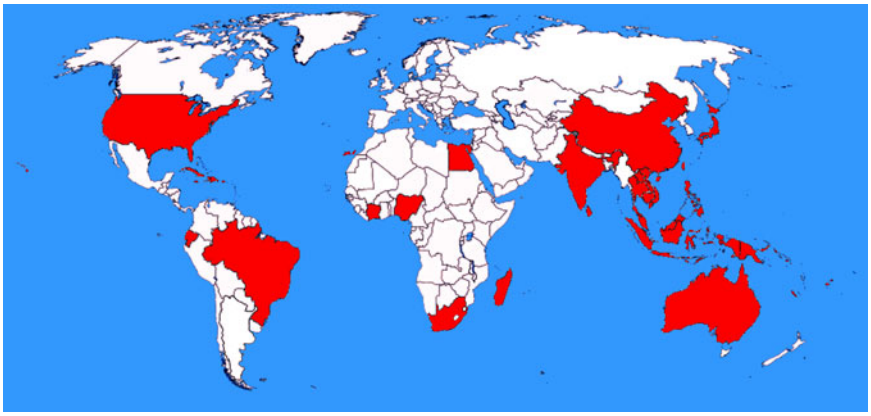

Fig. 5. Countries where *A. cantonensis* has been detected in naturally infected hosts. Shaded countries are those in which *A. cantonensis* was identified in studies screening naturally infected animals, or where humans have acquired infections. Unshaded countries include those that are yet to find evidence of *A. cantonensis*, or countries where studies examining potential hosts for *A. cantonensis* infection have not been carried out. This map does not include countries that have reported sporadic clinical cases of angiostrongyliasis that were probably acquired abroad. Mainland Australia is shaded on the map, though the island state of Tasmania is not. Native rats in Tasmania are known hosts of *A. mackerrasae* (Prociv *et al.* 2000), though *A. cantonensis* has not been reported in Tasmania. Mainland Spain is also not shaded on the map, although *A. cantonensis* was detected in the Canary Islands, off the western coast of Africa, which are part of Spain. Currently *A. cantonensis* is not known to be present in mainland Spain.

the predicted mode of transmission in two cases of severe *Angiostrongylus* eosinophilic meningitis (AEM) recently reported in young children (Morton *et al.* 2013). In these cases, a history of mollusc consumption was denied, though this is difficult to confirm in young children. Freshwater may represent another source of infection as *A. cantonensis* L_3 larvae reportedly survive in freshwater for up to 72 h (Cheng and Alicata, 1964). Studies of the feline metastrongyloid lungworms *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* demonstrated that L_3 larvae were also shed in mollusc mucus, and could be found in sediments from tap water containing *C. aspersum* that had been experimentally infected and drowned (Giannelli *et al.* 2015).

EPIDEMIOLOGY AND GLOBAL DISTRIBUTION

Angiostrongylus cantonensis is the most common cause of eosinophilic meningitis. It has been reported in over 30 countries since its identification as a human pathogen in 1945, predominantly in the tropics and sub-tropics (Beaver and Rosen, 1964; Wang *et al.* 2012; Eamsobhana *et al.* 2013*a*; Eamsobhana, 2014) (Tables 1 and 2, Fig. 5). While more than 2877 human cases of angiostrongyliasis have been described, many have probably gone unreported because of a lack of awareness and difficulties associated with its diagnosis (Qvarnstrom *et al.* 2007). Reports of angiostrongyliasis in Europe and other regions where it was not known previously are becoming more frequent, with travel to parts of Asia, the Pacific Islands and Latin America noted as the likely route of exposure for affected individuals (Bartschi *et al.* 2004; Chancellor, 2007; Leone *et al.* 2007; Ali *et al.* 2008; Malvy *et al.* 2008; Luessi *et al.* 2009; Maretic *et al.* 2009; Lammers *et al.* 2015).

While it is traditionally considered a disease of the Far East, reports of locally acquired angiostrongyliasis are becoming increasingly common in sub-tropical and temperate regions (Gutteridge *et al.* 1972; Prociv *et al.* 2000; Senanayake *et al.* 2003; Blair *et al.* 2013; Morton *et al.* 2013). It may be that the range of *A. cantonensis* has expanded recently, possibly as a consequence of global warming or other environmental factors (York *et al.* 2014). Hochberg *et al.* (2007) attribute the spread of *A. cantonensis* to the sheer diversity of its intermediate hosts, and the efficient dispersion of ship-borne rats. A similar expansion in geographic range has not been reported for *A. costaricensis*; the causative agent of human abdominal angiostrongyliasis (Spratt, 2015). The first documented human *A. costaricensis* infection was from Costa Rica (Morera and Cespedes, 1971; Morera, 1973), and later reports confirmed its presence in Venezuela, Ecuador, Honduras, Mexico, Nicaragua, Brazil, Guatemala, Columbia, the Caribbean Islands and Southern USA (Morera *et al.* 1983; Incani *et al.* 2007; Palominos *et al.* 2008; Spratt, 2015). Apparently, only one case of human *A. costaricensis* infection has been reported outside the Americas; an isolated case in an African man (Baird *et al.* 1987). The relatively restricted range of *A. costaricensis* is probably related to the limited range of its preferred definitive host; the hispid cotton rat (*Sigmodon hispidus*) (Graeff-Teixeira *et al.* 1990), which is generally found only in southern North America and parts of Central and South America.

Most human cases of angiostrongyliasis (~1300 cases; 47% of all cases worldwide) have been reported in Thailand (Wang *et al.* 2008), where between 0·3

and 2 people per 100 000 become infected annually (Suankratay *et al*. 2001; Eamsobhana, 2013). This high prevalence is attributable to the dietary habits of the local populace, involving the regular consumption of dishes like 'koi-hoi' that contain raw or undercooked molluscs such as *Pomacea maculata*, *P. canaliculata* and *Pila* spp.; common intermediate hosts of *A. cantonensis* (Schmutzhard *et al*. 1988; Lv *et al*. 2009*a*; Eamsobhana *et al*. 2010; Odermatt *et al*. 2010; Cowie, 2013*b*; Eamsobhana, 2014; Kim *et al*. 2014). The consumption of undercooked monitor lizard livers was linked to a fatal case of AEM in Thailand (Eamsobhana, 2014), where very high rates of monitor lizard infection have been reported; as high as 96% in one population (Eamsobhana, 2013). Angiostrongyliasis has also been reported in the neighbouring Southeast Asian Nations of Laos (Harinasuta, 1983), Cambodia (Brumpt *et al*. 1968) and Vietnam (Chau *et al*. 2003).

While the majority of AEM cases are reported from Thailand, most epidemiological data on angiostrongyliasis, including intermediate and definitive host surveys, comes from China. Several outbreaks of angiostrongyliasis have occurred in China, predominantly in provinces along the eastern coast (Lin *et al*. 2005; Wang *et al*. 2007, 2010; Lv *et al*. 2008, 2009*a*; Zhang *et al*. 2008*a*; Zhou *et al*. 2009; Hu *et al*. 2011). Only three cases of AEM were reported in China between 1984 and 1996, and the recent increase in cases is attributable to widespread changes in human dietary patterns, where snails have become a popular food item (Chen *et al*. 2005; Zhou *et al*. 2009; Wang *et al*. 2010). *Achatina fulica* and *P. canaliculata* are considered the most important species for *A. cantonensis* transmission in China, where both are introduced species, farmed as a food source (Wang *et al*. 2007; Lv *et al*. 2008, 2009*b*). Multiple Chinese outbreaks were directly linked to the consumption of raw or undercooked *P. canaliculata*, which is more popular as a Chinese food item than *A. fulica* (Wang *et al*. 2007; Lv *et al*. 2009*a*, *b*; Zhou *et al*. 2009). Consequently, *P. canaliculata* is aquacultured intensively for human consumption; it is often sold in local markets and served regularly in restaurants (Zhang *et al*. 2008*a*; Lv *et al*. 2009*a*, *b*; Zhou *et al*. 2009; Wang *et al*. 2010; Li *et al*. 2013*a*). *Pomacea canaliculata* is also more widespread in China than the terrestrial *A. fulica*, possibly because of its efficient spread via waterways during flooding (Lv *et al*. 2009*b*).

Serological surveys suggest human exposure to *A. cantonensis* is common in China. On Hainan Island, 92 of 459 subjects (20·04%) had antibodies to *A. cantonensis* (Hu *et al*. 2011). In another study from Hainan Island, anti-*A. cantonensis* immunoglobulin G (IgG) was detected in 20·6% of 393 participants, 12·5% of whom habitually ate raw snails (Li *et al*. 2011). In Guangdong Province, 42 of 300 people (14%) had IgG antibodies to *A. cantonensis*, five of whom had been recently exposed based on the detection of circulating IgM (Zhang *et al*. 2008*b*). The prevalence of *A. cantonensis* on Hainan Island was equal for both genders, though subjects under the age of 14 were more likely to be seropositive (Hu *et al*. 2011). Chen *et al*. (2011*c*) detected circulating *A. cantonensis* antigen at a prevalence of 0·8% in members of the general Chinese population though differences were observed between certain groups. Males had a higher prevalence than females and those involved in the aquaculture or processing of molluscs for human consumption were more likely to have circulating antigen (Chen *et al*. 2011*c*). The prevalence of *A. cantonensis* in molluscs and rats has also been investigated in several regions in China (Table 2).

Hundreds of AEM cases have been reported in Taiwan, many of which were linked to the handling or consumption of *P. canaliculata* and *A. fulica* (Hwang and Chen, 1991; Tsai *et al*. 2004, 2013; Wan and Weng, 2004; Wang *et al*. 2007). The aquatic snail, *Bellamya chinensis*, is also considered an important intermediate host of *A. cantonensis* in Taiwan (Lv *et al*. 2009*b*). Early Taiwanese reports of AEM described cases predominantly in children and indigenous Taiwanese (Yii *et al*. 1975; Hwang and Chen, 1991), though recent reports more commonly describe infections in adults (Tseng *et al*. 2011, 2013). In those earlier cases, infections peaked during the rainy season, and those affected often reported eating thoroughly cooked *A. fulica* prior to disease (Yii *et al*. 1975). This suggests that *A. cantonensis* may have been ingested inadvertently during preparation of the snails for cooking.

Cases of AEM in Hawaii have been reported from as early as 1958 (Wallace, 2013). Between 2001 and 2005, 24 cases were reported though only one case was parasitologically confirmed; a case involving a young child. Most of these cases occurred in Honolulu, on the main island of Hawaii (Hochberg *et al*. 2007, 2011). Deliberate consumption of raw or undercooked molluscs is rare in Hawaii and most exposures were attributed to inadvertent ingestion of molluscs or their mucus. Patients sometimes recalled finding slugs in their food or drink, and the mother of another patient recalled her infant child putting grass in her mouth (Hochberg *et al*. 2011). *Parmarion martensi* is considered important for maintaining the endemicity of *A. cantonensis* in Hawaii, given its high prevalence in Hawaiian populations, and the remarkably high parasite burdens observed in some specimens (Tables 1 and 2).

In Australia, virtually all native and introduced terrestrial and freshwater molluscs can be experimentally infected with *A. cantonensis* (Prociv and Carlisle, 2001). Despite this, the frequency of infected molluscs in Australia is low compared to

what is observed in Southeast Asia (Chan *et al.* 2015). It was suggested that the mollusc species in Australia may not be the preferred hosts of *A. cantonensis* (Chan *et al.* 2015). Furthermore, the climate in Sydney is temperate to sub-tropical, whereas transmission of *A. cantonensis* is favoured by tropical climates which experience warm temperatures, high humidity and high rainfall (Hu *et al.* 2011). The minimum temperature threshold (the temperature at which larvae halt development in their mollusc hosts), is approximately 15 °C for *A. cantonensis* (Lv *et al.* 2006; Morley, 2010). In Sydney, temperatures regularly fall below 15 °C in winter, probably restricting *A. cantonensis* transmission to the warmer months of spring and summer. In Eastern Australia the prevalence of *Angiostrongylus* spp. in *Rattus* spp. is high compared with its prevalence in molluscs (Table 2).

Human cases of AEM have been diagnosed in multiple cities along the eastern coast of Australia including Brisbane, Sydney and Melbourne (Prociv *et al.* 2000). The first Australian case of human AEM was reported in 1971 in the Brisbane region (Gutteridge *et al.* 1972), and several human cases have been reported in Australia since (Prociv and Carlisle, 2001; Senanayake *et al.* 2003; Blair *et al.* 2013; Morton *et al.* 2013). Australian clinical cases mostly involved young children or infants, with or without a known history of mollusc consumption (Prociv *et al.* 2000; Morton *et al.* 2013). Two cases involved young adults who knowingly ingested slugs from the Sydney area (Senanayake *et al.* 2003; Blair *et al.* 2013). The rate of human infections is proportionally lower in Australia compared with China and Thailand, probably because snails are not widely eaten in Australia. Given the rarity of human angiostrongyliasis in Australia and its mostly self-limited nature, some infections have probably gone unreported. Regardless, the recent cases of severe angiostrongyliasis and increasing range of *A. cantonensis* underpin the need for increased awareness of angiostrongyliasis in Australia, particularly given that the aetiological agent sometimes remains unidentified until post-mortem (Morton *et al.* 2013).

Angiostrongylus cantonensis has also been detected in Japan, parts of Southern USA, the Caribbean Islands [though it was absent in rats from Barbados (Levett *et al.* 2004)], Tenerife, Brazil, Papua New Guinea, French Polynesia, Fiji, the Philippines, Indonesia, Sri Lanka, India, South Africa and Egypt (Tables 1 and 2, Fig. 5) (Alicata, 1965*b*; Kliks and Palumbo, 1992; Uga *et al.* 1996; Asato *et al.* 2004; Batmanian and O'Neill, 2004; Lindo *et al.* 2004; Abo-Madyan *et al.* 2005; Owen, 2005; Waugh *et al.* 2005; Caldeira *et al.* 2007; Chikweto *et al.* 2009; Dorta-Contreras *et al.* 2009; Archer *et al.* 2011; Constantino-Santos *et al.* 2014; Oehler *et al.* 2014; Okano *et al.* 2014; Lammers *et al.* 2015; Stockdale-Walden *et al.* 2015).

PATHOPHYSIOLOGY AND CLINICAL MANIFESTATIONS

Angiostrongylus cantonensis larvae are neurotropic, preferentially infecting CNS tissue, and causing inflammation at these sites. Autopsies of AEM patients indicate that the external surfaces of the brain and the spinal cord are generally normal and gross haemorrhage is uncommon, though has been reported in severe human cases (Wang *et al.* 2008; Morton *et al.* 2013). Infiltration of lymphocytes, plasma cells and eosinophils is common in the meninges (Wang *et al.* 2008) (Fig. 6). Third-stage larvae may be observed in association with the meninges and nerve roots (Chen *et al.* 2005). Eosinophilic pleocytosis in the CSF and increased CSF protein are common (Schmutzhard *et al.* 1988; Dorta-Contreras *et al.* 2011). Cellular infiltration around live worms is unusual though dead worms precipitate granuloma formation, infiltration by eosinophils and occasionally Charcot–Leyden crystals (Wang *et al.* 2008; Martins *et al.* 2015). Physical tracks and microcavities due to the burrowing movement of larvae may be observed in the brain and spinal cord (Morton *et al.* 2013; Murphy and Johnson, 2013).

Angiostrongyliasis initiates a Th2 immune response in the CNS, characterized by the infiltration of eosinophils into the subarachnoid space, and CSF eosinophilia (Intapan *et al.* 2008; Graeff-Teixeira *et al.* 2009; Murphy and Johnson, 2013; Martins *et al.* 2015). The role of eosinophils in the immune response to helminth infections is incompletely understood (Klion and Nutman, 2004). However, the classical view of eosinophils as effectors for killing worms has given way to the idea that they are probably not directly involved in killing them, but are actually important regulators of the cellular immune response (Gebreselassie *et al.* 2012; Gosnell and Kramer, 2013). As supported by rodent studies of AEM, microglia excrete eosinophil chemoattractants such as eotaxin and macrophage inflammatory protein in response to dead worms, to recruit eosinophils to the brain parenchyma (Chang *et al.* 2004; Chang and Yen, 2004; Intapan *et al.* 2007; Gosnell and Kramer, 2013; Zhao *et al.* 2013; Li *et al.* 2014*a*; Wei *et al.* 2015). Once in the brain, eosinophils enhance this Th2 response by producing Th2 cytokines, excreting chemoattractants, and presenting antigen to $CD4^+$ T cells (Spencer and Weller, 2010). Consequently, levels of Th2 cytokines such as interleukin (IL) 4, IL5, IL10 and IL13 increase in the brain and CSF (Intapan *et al.* 2008; Yu *et al.* 2015). Th2 cytokines may also become elevated in the periphery (Diao *et al.* 2009). Angiostrongyliasis can increase CNS levels of IL33; an important mediator of eosinophil infiltration, Th2 cell differentiation and expression of IL5 and IL13 (Du *et al.* 2013; Peng *et al.* 2013;

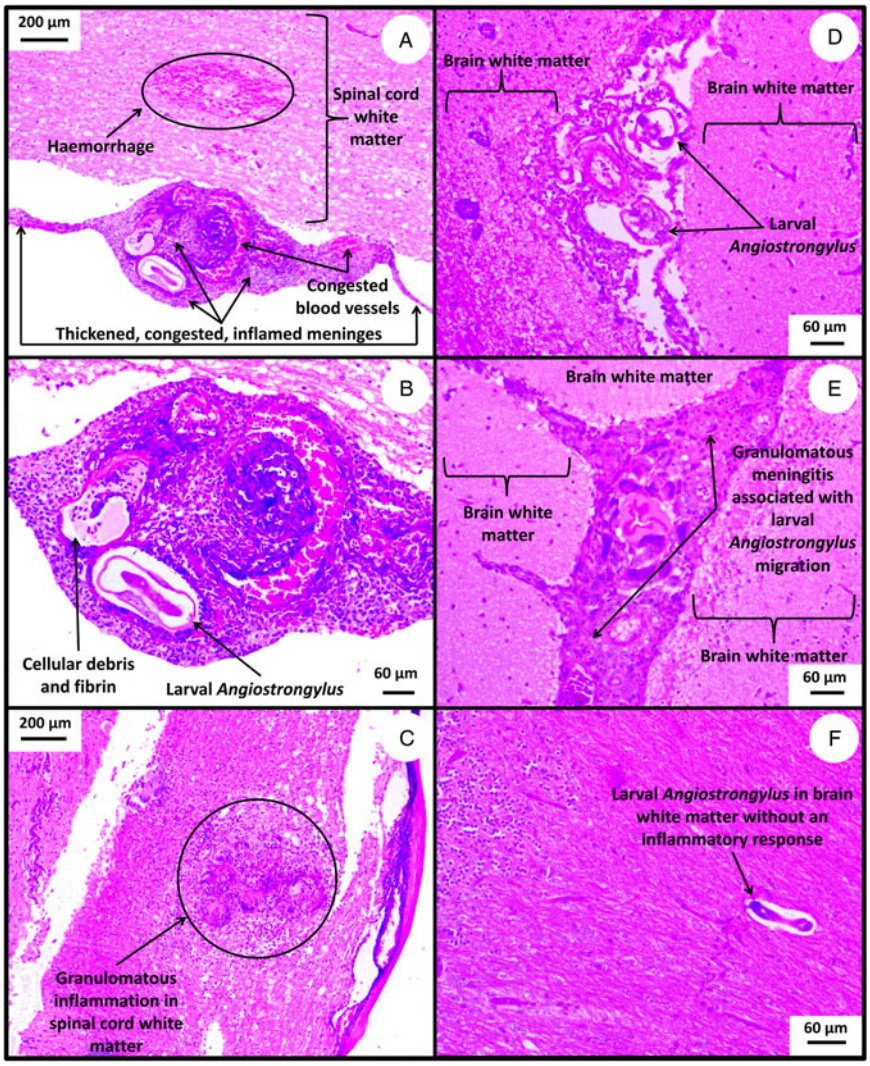

Fig. 6. Haematoxylin and eosin-stained tissue sections from Australian wildlife showing histopathological changes caused by *A. cantonensis* infection. (A) Spinal cord section from a sub-adult female brushtail possum that had hind limb paralysis. Focal haemorrhage of the spinal cord is apparent, along with greatly thickened, congested and inflamed meninges. (B) The same spinal cord section shown in (A), though at a higher magnification. The focal haemorrhage and thickened, congested, inflamed meninges can be seen more clearly, associated with a cross-section of a larval nematode identified as *A. cantonensis* in this animal. The meninges are infiltrated by plasma cells, lymphocytes, macrophages and neutrophils (marked non-suppurative meningitis). (C) Spinal cord from the same brushtail possum showing tissue damage and granulomatous inflammation in the white matter where presumably a larval *Angiostrongylus* travelled through. (D) Brain section from an affected tawny frogmouth showing cross-sections of larval *Angiostrongylus* associated with granulomatous meningitis. (E) Brain and meninges from the same tawny frogmouth as (D), showing more obvious granulomatous meningitis. (F) Brain from another affected tawny frogmouth showing a larval *Angiostrongylus* migrating through the white matter before an inflammatory response has occurred.

Chuang *et al*. 2016; Saluja *et al*. 2015). IL5 and IL13 are two of several important regulators of IgE production (Deo *et al*. 2010). As a result, intrathecal IgE may be elevated in AEM (Dorta-Contreras *et al*. 2005, 2011; Padilla-Docal *et al*. 2008). Monocyte chemotactic protein 1 is also expressed in the brain of mice in response to injury caused by migrating larvae (Yu *et al*. 2015).

Blood brain barrier (BBB) dysfunction is a feature of AEM, associated with the activity of host matrix metalloproteinase-9 (MMP-9); a protease that degrades extracellular matrix proteins such as fibronectin and elastin (Hsu and Lai, 2007; Wei *et al*. 2011). In healthy brain tissue, MMP-9 expression is low, though becomes elevated in response to certain stimuli, including brain tissue damage (Vafadari *et al*. 2016). In AEM, MMP-9 expression increases, possibly due to the damage inflicted by migrating worms (Tsai *et al*. 2008). Mouse studies of AEM suggest that eosinophils release MMP-9 into the subarachnoid space (Tseng *et al*. 2004), activating a proteolytic cascade that disrupts the BBB (Chen *et al*. 2006; Tsai *et al*. 2008; Chiu and Lai, 2013, 2014). In mice with AEM, MMP-9 was also

observed within endothelial cells lining the vascular spaces of the brain and in leucocytes within the subarachnoid space (Lai *et al*. 2004). *Angiostrongylus cantonensis* also excretes MMPs and other proteases involved in the pathogenesis of AEM (discussed in a later section). Dysfunction of the BBB in AEM may also be mediated by vascular endothelial growth factor; an inducer of vascular permeability and mediator of brain oedema (Tsai *et al*. 2007*a*). Levels of pro-apoptotic proteins, plasminogen activators, and reactive oxygen species are also elevated in the CNS of mice with AEM (Hou *et al*. 2004; Chen *et al*. 2006, 2008). Expression of the 14-3-3β protein; a marker of neuronal damage, is also elevated in CSF and serum from AEM patients (Tsai *et al*. 2014*a*).

The clinical manifestations of angiostrongyliasis occur partly as a result of increased intracranial pressure (ICP); a common clinical sign of AEM (Graeff-Teixeira *et al*. 2009; Murphy and Johnson, 2013). Increased ICP may result from vasodilation in the subarachnoid space and brain parenchyma, decreased absorption of CSF, or brain oedema (Murphy and Johnson, 2013). As a consequence of high ICP, AEM patients often present with mild to severe headaches, though prolonged high ICP can eventuate in more serious neurological sequelae (Wang *et al*. 2008, 2010; Tseng *et al*. 2011; Murphy and Johnson, 2013).

Eosinophilic meningitis is the most common manifestation of angiostrongyliasis (Wang *et al*. 2012). Rarely, severe sequelae including coma, convulsion, epilepsy, amentia, hypomnesia and even death can occur (Wang *et al*. 2010; Howe, 2013; Morton *et al*. 2013). However, patients more often present with headache, neck stiffness, paraesthesia, muscle weakness, Brudzinski's sign, fever, vomiting and nausea. Symptoms such as face/limb paralysis, memory loss, confusion, dizziness, conscious disturbance, tinnitus, hyperesthesia, dystonia, urinary retention, photophobia, pneumonitis, peritonitis, abdominal pain, bowel dysfunction, orbital/retro-orbital pain and paralysis of the extra-ocular muscles, are also less common (Chau *et al*. 2003; Podwall *et al*. 2004; Furugen *et al*. 2006; Jin *et al*. 2008; Li *et al*. 2008; Wang *et al*. 2008, 2010; Tseng *et al*. 2011; Kwon *et al*. 2013). Symptoms can be protracted, taking months to disappear (Hochberg *et al*. 2011). In humans and animals, neurological damage is sometimes irreversible (Chau *et al*. 2003; Batmanian and O'Neill, 2004) (Fig. 4, online Supplementary materials). Encephalitic angiostrongyliasis is a rarer manifestation that is generally fatal (Sawanyawisuth, 2008). Elderly patients who become infected with *A. cantonensis* and experience fever and prolonged headaches are at greater risk of developing encephalitic angiostrongyliasis (Sawanyawisuth, 2008; Sawanyawisuth *et al*. 2009).

Myelitis, sacral myeloradiculitis and inflammation of the nerve roots can occur in AEM (Hsu *et al*. 2009; Murphy and Johnson, 2013; Ueda *et al*. 2015), though this is more often associated with gnathostomiasis; the disease caused by *Gnathostoma* spp. worms (Schmutzhard *et al*. 1988). Intraparenchymal cerebral haemorrhage has also been reported (Lilic and Addison, 2013). Presumably, disease severity and incubation period vary depending on the number of larvae consumed (Murphy and Johnson, 2013). Incubation periods range from as little as 1 day to several months, with a median of 11 days following ingestion of L_3 larvae (Zhou *et al*. 2009; Tseng *et al*. 2011; Wang *et al*. 2012; Murphy and Johnson, 2013; Sawanyawisuth *et al*. 2013). As human angiostrongyliasis is often self-limiting, mortality rates are usually low (Wang *et al*. 2008; Graeff-Teixeira *et al*. 2009).

While there is little supporting evidence, children seem especially predisposed to angiostrongyliasis, as evidenced by their disproportionate representation in some outbreaks (Yii, 1976), and the particularly severe cases reported in infants and children (Prociv *et al*. 2000; Li *et al*. 2001; Lindo *et al*. 2004; Morton *et al*. 2013; Murphy and Johnson, 2013; Evans-Gilbert *et al*. 2014). The reason for this remains unclear. However, in cases such as those reported from Taiwan, infected children were thought to have been playing with live snails and possibly eating them (Yii *et al*. 1975; Yii, 1976), which is something adults are unlikely to do. This may expose children to greater numbers of larvae, resulting in severer manifestations. Wang *et al*. (2008) and Sawanyawisuth *et al*. (2013) also note that the clinical manifestations of AEM differ between adults and children; somnolence, fever, constipation, abdominal pain, vomiting, nausea, hepatomegaly, neck stiffness and cranial nerve palsies were more common in children.

In approximately 1·1% of cases *A. cantonensis* causes ocular disease, which may or may not be concurrent with AEM (Patikulsila *et al*. 2003; Sawanyawisuth *et al*. 2007; Baheti *et al*. 2008; Chi *et al*. 2014). Patients with ocular angiostrongyliasis may present with blepharospasm, diplopia, strabismus, blurred vision, loss of colour vision or complete vision loss (Liu *et al*. 2006; Wang *et al*. 2006*b*; Sawanyawisuth *et al*. 2007; Sinawat *et al*. 2008; Qi *et al*. 2009). The route taken by *A. cantonensis* to enter the eye is unknown, though worms may travel from the brain via the optic nerve, through the circulation via the retinal artery, or enter the eye directly from the environment (Martins *et al*. 2015). Worms may be found in the anterior chamber, the vitreous cavity or the subretinal space (Kumar *et al*. 2005; Malhotra *et al*. 2006; Sawanyawisuth *et al*. 2007; Crane *et al*. 2013; Sinawat and Yospaiboon, 2013; Galor and Eberhard, 2014). Clinical signs of ocular angiostrongyliasis are diverse and may include uveitis, macular

oedema, retinal oedema, necrotic retinitis, panophthalmitis, papilledema, optic neuritis, optic nerve compression, orbital inflammation, increased intraocular pressure, retinal oedema, macular oedema and a pale optic disc (Kumar *et al*. 2005; Liu *et al*. 2006; Wang *et al*. 2006*b*; Sawanyawisuth and Kitthaweesin, 2008; Sinawat *et al*. 2008; Qi *et al*. 2009; Feng *et al*. 2013; Sinawat and Yospaiboon, 2013; Chi *et al*. 2014). Altered epithelial pigment and subretinal tracks may also be observed (Sinawat *et al*. 2008).

DIAGNOSIS

Diagnosis of AEM is often overlooked, particularly in regions previously considered non-endemic. The lack of standardization in diagnostic procedures for angiostrongyliasis often results in a presumptive diagnosis; based on patient history and suggestive clinical findings (i.e. eosinophilic meningitis) (Murphy and Johnson, 2013). A history of residence or travel to endemic regions and/or a history of raw mollusc consumption are integral in establishing the diagnosis (Tsai *et al*. 2003; Cowie, 2013*b*). A history of eating unwashed fresh produce, such as lettuce, is also informative (Lindo *et al*. 2002; Waugh *et al*. 2005). An accurate patient history may differentiate AEM from neural gnathostomiasis, which can also manifest as eosinophilic meningitis. While AEM patients often have a history of eating raw molluscs, gnathostomiasis patients usually recall eating undercooked poultry or fish (Senthong *et al*. 2013).

Microscopic detection of L_3 larvae (Fig. 2) from a patient's CSF or eye provides a definitive diagnosis. However, the sensitivity of CSF microscopy depends on sample volume, which is generally limited, often leading to poor sensitivity and false negative results (Prociv *et al*. 2000; Chen *et al*. 2005; Graeff-Teixeira *et al*. 2009). Molecular and immunological tests offer greater sensitivity and several of these have been described for angiostrongyliasis (discussed in later sections). Peripheral blood and CSF eosinophil counts, and CT or MRI imaging can aid the diagnosis (Graeff-Teixeira *et al*. 2009). In ocular angiostrongyliasis, worms may be observed by slit lamp examination of the eye (Malhotra *et al*. 2006; Mattis *et al*. 2009).

During a helminth infection, the proportion of eosinophils may reach 7–36% of the total white blood cell count in peripheral blood (normal range is 0·5–5%) and 10% or more (100–1000 eosinophils per μL) of the white cell count in CSF (normal range is <10 eosinophils/μL^{-1}) (Schulte *et al*. 2002; Wang *et al*. 2008; Sawanyawisuth and Chotmongkol, 2013). Eosinophils can be difficult to differentiate from neutrophils using some staining techniques such as the toluidine blue wet film. In aseptic meningitis, particularly associated with peripheral eosinophilia, more specific Romanowsky-based stains such as the May–Grünwald–Giemsa stain or Wright stain should be performed (Senanayake *et al*. 2003; Graeff-Teixeira *et al*. 2009). The Diff-Quik stain may also be useful, having been used to confirm eosinophilia in cases of canine angiostrongyliasis (Lunn *et al*. 2012). While *A. cantonensis* is the leading cause of eosinophilic meningitis, other aetiological agents must be considered in the differential diagnosis (Table 3).

Brain MRI or CT scans may reveal some abnormalities in AEM patients, though lesions resulting from gnathostomiasis are often distinct from those seen in AEM (Senthong *et al*. 2013). In gnathostomiasis with CNS involvement, abnormal CT or MRI findings are common (Kanpittaya *et al*. 2012). Conversely, AEM patients may have normal CT or MRI findings though non-specific cerebral oedema, focal oedematous changes, nodular enhancing lesions, meningeal/leptomeningeal enhancement and mild ventricular dilatation may be apparent (Tsai *et al*. 2003, 2012; Jin *et al*. 2008; Wang *et al*. 2010; Tseng *et al*. 2011; Kanpittaya *et al*. 2012; Nalini *et al*. 2013). Intracerebral haemorrhage and myelitis are more suggestive of gnathostomiasis (Kanpittaya *et al*. 2012). Lesions may also be present on the spinal cord of AEM patients (Diao *et al*. 2010). Brain abnormalities may become apparent in MRI scans as early as 3 weeks after the onset of AEM symptoms (Jin *et al*. 2005).

Immunodiagnostic assays

Immunodiagnostic tests for angiostrongyliasis using purified antigens or monoclonal antibodies have been available for decades. The earliest enzyme-linked immunosorbant assays (ELISAs) were developed using crude or partially purified adult *A. cantonensis* antigens (Chuan-Min and Eng-Rin, 1991). Two immunodominant *A. cantonensis* antigens were identified; a 29 and a 31 kDa antigen, and most immunoassays described target these (Wilkins *et al*. 2013) (Table 4). Immunoblots targeting the 29 kDa antigen cross-reacted with sera from patients infected with other tissue-invading helminths, while tests targeting the 31 kDa antigen exhibited greater specificity (Wilkins *et al*. 2013).

As most cases of AEM originate in Thailand, routine serological testing has been implemented there in several regional hospital laboratories (Eamsobhana and Yong, 2009). These laboratories utilize an in-house dot-blot assay based on the 31 kDa antigen, purified from *A. cantonensis* worms (Eamsobhana *et al*. 2003, 2006; Eamsobhana, 2013; Wilkins *et al*. 2013). This 31 kDa antigen consists of up to four distinct glycoproteins that react to sera from angiostrongyliasis patients (Morassutti *et al*. 2012*a*). As purification of these glycoproteins from worms is laborious, attempts were made to

Table 3. Causative agents to be considered in the differential diagnosis of eosinophilic meningitis

Disease	Aetiology/causal species
Parasitic infections	
Gnathostomiasis	*Gnathostoma spinigerum*
Schistosomiasis	*Schistosoma mansoni Schistosoma haematobium, Schistosoma japonicum, Schistosoma mekongi, Schistosoma malayensis*
Cysticercosis	*Taenia Solium*
Toxocariasis	*Toxocara canis, Toxocara cati*
Baylisascariasis	*Baylisascaris procyonis*
Paragonimiasis	*Paragonimus miyazakki, Paragonimus skrjabini Paragonimus heterotremus, Paragonimus africanus, Paragonimus uterobilateralis, Paragonimus kellicoti, Paragonimus mexicanus, Paragonimus westermani*
Trichinellosis	*Trichinella spiralis*
Hydatidosis	*Enchinococcus granulosus, Enchinococcus multilocularis*
Coenurosis	*Taenia multiceps*
Strongyloidiasis	*Strongyloides stercoralis*
Filariasis	*Loa loa, Wucheria bancrofti, Onchocerca volvulus, Meningonema peruzzi*
Arthropod infections	
Myasis	Maggots
Fungal Infections	
Coccidioiomycosis	*Coccidiodides immitis* *Coccidioides posadasii*
Allergic aspergillus sinusitis	*Aspergillus* spp.
Bacterial Infections	
Streptococcal newborn infection	Group B Streptoccocal Infection
Neurosyphilis	*Treponema pallidum*
Rocky Mountain spotted fever, Ricketsial illness	*Rickettsia* spp.
Viral infections	
Coxsackie viral meningitis	Coxsackie virus
Lymphocytic choriomeningitis	*Arenaviridae*
Other causes of eosinophilic meningitis	
Neoplasms	Chronic Eosinophilic Leukaemia, Systemic Mastocytosis, Chronic Myelomonocytic Leukaemia, Juvenile Myelomonocytic Leukaemia, Chronic Myeloid Leukaemia, Atypical Chronic Myeloid Leukaemia, Idiopathic Hypereosinophilic Syndrome
Drugs	Ibuprofen, Ciprofloxacin, Vancomycin, Gentamicin, Rifampicin, Minocycline
Reagents and plastic devices	Intravenous Catheters, Intracranial Hardware, Iodized oils, Illicit drugs

Note: This table was prepared using information presented by Graeff-Teixeira *et al*. (2009) and Diaz (2009).

generate recombinants of them for immunodiagnostic purposes (Morassutti *et al*. 2013*a*). This endeavour has been met with limited success so far, which may be attributable to the choice of expression system (Morassutti *et al*. 2013*a*). Eamsobhana *et al*. (2015*b*) recently described a rapid dot-immunogold filtration assay for detecting serum antibodies reactive to the 31 kDa antigen. This assay boasts 100% sensitivity and specificity and produces a result in 3–5 min, compared to the 3 h required for an immunoblot test result (Eamsobhana *et al*. 2015*b*).

Currently, Shenzhen Combined Biotech Co. Ltd, China, provides the only commercial immunoassays for detecting anti-*A. cantonensis* antibodies, though these have not been implemented for routine diagnostic use based on current literature. The company produces three ELISA tests; one for detecting human IgG, another for detecting human IgM, and a third for detecting rat IgG (Hu *et al*. 2011).

Molecular assays

Several nucleic acid amplification assays have been described for the detection of *A. cantonensis* DNA, most targeting the ribosomal RNA genes (rDNA) (Table 4). Given the limited genetic variation in the rDNA between the various Angiostrongylidae, new tests that differentiate them with improved resolution must focus on different markers (Chan *et al*. 2015). The development of molecular tests that differentiate closely related Angiostrongylidae was initially hampered by the limited availability of sequence data for them. A technique for distinguishing certain *Angiostrongylus* spp. was developed several years ago; a PCR restriction fragment length polymorphism technique targeting the mitochondrial *cytochrome c oxidase subunit I* (*COI*) gene, and the internal transcribed spacer 2 (ITS2) DNA (Caldeira *et al*. 2003). It is unknown however, whether this technique can differentiate between the closely related *A. cantonensis* and *A. mackerrasae*. With the current

Table 4. Assays recently evaluated for the specific detection of *A. cantonensis*

Diagnostic method	Target	Sensitivity	Specificity	Reference
Microscopy				
Microscopy of CSF	Third-stage larvae	Low – dependent on the volume of CSF tested	High	Graeff-Teixeira *et al.* (2009); Rai *et al.* (2014)
Immunological				
Rapid dot-immunogold - filtration assay	Detects serum antibodies against purified 31 kDa glycoprotein of *A. cantonensis* in 3–5 min	100%	100%	Eamsobhana *et al.* (2014, 2015*b*)
Protein Microarray	Serum antibodies to semi-purified antigens of *A. cantonensis*	92·1%	100%	Chen *et al.* (2012*b*)
ELISA	Serum antibodies to semi-purified antigens of *A. cantonensis*	92·1%	100%	Chen *et al.* (2012*b*)
Sandwhich ELISA	Excreted/secreted antigens from adult *A. cantonensis* in CSF and serum	100% – Minimum detectability was 0·5 ng of antigen per ml of serum	100% – No cross-reactivity with sera from patients with any of nine other parasites	Chen *et al.* (2011*b*)
Immunoblot	Serum antibodies to a 29 kDa antigen of *A. cantonensis*	55·6%	100%	Sawanyawisuth *et al.* (2011)
Gold immuno-chromatography	A 55 kDa protein from adult worms of *A. cantonensis* in patient sera	100%	100% – No cross-reactivity with sera from patients with any of five other parasites	Huang *et al.* (2010)
Dot-blot ELISA	Serum antibodies to purified crude antigen of *A. cantonensis*	81·3% of eosinophilic meningitis patients tested positive	100% – No cross-reactivity with sera from patients with other parasites ($n = 18$) or patients with other causes of meningitis ($n = 52$)	Tomanakan *et al.* (2008)
ELISA	CSF antibodies of different IgG subclasses to young adult female *A. cantonensis* antigen	13–47% – IgG subclass dependent	90–100% – IgG subclass dependent	Kittimongkolma *et al.* (2007)
Dot-blot assay	Serum antibodies to antigens between 3 and 100 kDa from adult *A. cantonensis*	100%	86%	Eamsobhana *et al.* (2006)
Sandwich ELISA	Circulating antigen of adult *A. cantonensis* in patient sera	86·4%	100% – No cross-reactivity with sera from patients with any of four other parasites	Liang *et al.* (2005)
Immuno-PCR	Circulating antigen from L_5 stage *A. cantonensis* in patient sera	98%	100%	Chye *et al.* (2004)
Dot-blot assay	Serum antibodies to a 31 kDa glycoprotein antigen from adult *A. cantonensis*	100%	100%	Eamsobhana *et al.* (2003, 2004)
Immunoblot	Serum antibody of the IgG4 class to a 29 kDa from young adult somatic extracts of *A. cantonensis*	75%	95%	Intapan *et al.* (2003)
ELISA	Antibodies to somatic antigen from young adult, female *A. cantonensis*	0–100% – Antibody class dependent; 100% for IgG1	88·9–100% – Antibody class dependent; 100% for IgG1	Intapan *et al.* (2002)

Table 4. (*Cont.*)

Diagnostic method	Target	Sensitivity	Specificity	Reference
Immunoblot	CSF and serum IgG to a 29 kDa antigen from young adult female *A. cantonensis*	55·6%	99·4%	Maleewong *et al.* (2001)
ELISA	Antibodies to a 204 kDa protein antigen from sub-adult (L_5) *A. cantonensis*	91% in sera and 83% in CSF	98% in sera and 100% in CSF	Chye *et al.* (2000)
Nucleotide amplification				
Real-Time PCR (with high-resolution melt curve)	ITS1 DNA	A single *A. cantonensis* L_3 larvae	No cross-reactivity with DNA from *Clonorchis sinensis* and *Gnathostoma spinigerum*	Ke *et al.* (2014)
Real-time PCR (TaqMan)	ITS2 DNA	At least a single L_1 larvae in a grain of rat feces	No cross-reactivity with DNA from four other rat parasites	Fang *et al.* (2012)
LAMP[a]	18S rRNA gene	1 fg of DNA	No cross-reactivity with DNA from six other parasites	Chen *et al.* (2011*d*)
LAMP[a]	ITS1 DNA	0·01 ng of DNA (0·32 larva per 0·1 g of snail tissue)	No cross-reactivity with DNA from five other parasites	Liu *et al.* (2011)
Multiplex PCR	18S rRNA gene	93·8%	80·6%	Wei *et al.* (2010)
Real-time PCR (TaqMan)	ITS1	Ten plasmid copies	No cross-reactivity with several DNA samples containing DNA from other parasitic and free living nematodes	Qvarnstrom *et al.* (2010)
Conventional PCR	18S rRNA gene	At least one L_3 larva per mg of mollusc tissue	Cross-reactivity reported with DNA from another metastrongyloid nematode	Qvarnstrom *et al.* (2007)
Conventional PCR	*A. cantonensis* gene sequence encoding a 66 kDa protein	55% – 18·5 ng μL^{-1} genomic DNA from *A. costaricensis*	100% – Failed to amplify DNA from CSF samples from patients with cerebral gnathostomiasis and neurocysticercosis	da Silva *et al.* (2003); Eamsobhana *et al.* (2013*b*); Rodriguez *et al.* (2014)

Note: This table only lists assays described in the literature during or after the year 2000. For information on earlier assays see the reviews by Graeff-Teixeira *et al.* (2009), Eamsobhana and Yong (2009) and Wilkins *et al.* (2013).
[a] LAMP, Loop-mediated isothermal amplification.

availability of several *Angiostrongylus* spp. mitochondrial genomes (discussed in a later section), the development of improved, species-specific assays may now be achievable. Molecular detection, though currently not used routinely, holds potential for the future diagnosis of angiostrongyliasis, enabling specific detection of *A. cantonensis* DNA in patient CSF (Wilkins *et al.* 2013; Qvarnstrom *et al.* 2016).

One conventional PCR assay amplifying a 1134 bp fragment of the 18S rDNA was used to detect *A. cantonensis* DNA in molluscs from Hawaii, in response to an angiostrongyliasis outbreak (Qvarnstrom *et al.* 2007). Sequencing of PCR products obtained in that study confirmed three false positives resulting from cross-reactivity with other nematode species; a consequence of the highly conserved nature of 18S rDNA (Qvarnstrom *et al.* 2007). Another PCR assay targeting a 66 kDa protein from *A. cantonensis/A. costaricensis* is also available. This assay detected *A. costaricensis* DNA in patient sera and in paraffin-embedded tissues (da Silva *et al.* 2003; Rodriguez *et al.* 2014). It also detected *A. cantonensis* DNA in patient CSF, though with comparatively low sensitivity (Table 4) (Eamsobhana *et al.* 2013*b*).

A TaqMan qPCR assay was developed for detecting *A. cantonensis* DNA in molluscs collected in the field in Hawaii (Qvarnstrom *et al.* 2010). This assay amplifies the ITS1 DNA region, which is less conserved than the 18S rDNA among helminth species (Qvarnstrom *et al.* 2010). The assay has also been

trialed on human CSF for diagnosing AEM, with promising results (Thyssen *et al*. 2013; Qvarnstrom *et al*. 2016). However, some patients returned intermittently positive and negative results, indicating that the amount of *A. cantonensis* DNA in CSF changes over the course of infection (Qvarnstrom *et al*. 2016). It has also been used to screen molluscs in field studies from various other locations (Tables 1 and 2), and to detect *A. cantonensis* DNA in the blood and peripheral tissues of experimentally infected, wild-type *R. rattus* from Hawaii (Jarvi *et al*. 2015). However, this assay was recently found to cross-react with DNA from *A. mackerrasae* (Chan *et al*. 2015). Consequently, a more specific test is required to confirm the prevalence of *A. cantonensis* in Australia, where *A. mackerrasae* is endemic (Mackie *et al*. 2013). *Angiostrongylus mackerrasae* causes disease in animals though its zoonotic potential remains unknown (Spratt, 2015). Some human cases of angiostrongyliasis may have been attributable to *A. mackerrasae* in Australia, though the absence of an assay that differentiates it from *A. cantonensis* makes this possibility difficult to explore. Mitochondrial genes such as the *COI* gene and *NADH dehydrogenase subunit 6* gene represent possible targets for differentiating closely related Angiostrongylidae as they possess greater variability between populations compared with the chromosomal rDNA sequences (Gasser *et al*. 2012; Lv *et al*. 2012, 2014; Tokiwa *et al*. 2012; Aghazadeh *et al*. 2015*c*; Yong *et al*. 2015*c*).

Two loop-mediated isothermal amplification (LAMP) assays have been developed for *A. cantonensis*, one targeting the ITS1 DNA (Liu *et al*. 2011) and the other targeting the 18S rDNA (Chen *et al*. 2011*d*). These assays were up to ten times more sensitive than conventional PCR (Chen *et al*. 2011*d*; Liu *et al*. 2011) and have the added benefit of being performed at a single temperature (~65 °C), on a simple heat block or in a warm water bath.

MOLECULAR BIOLOGY

Several wide-scale genomic, transcriptomic and proteome studies have contributed to our current understanding of *Angiostrongylus* spp. molecular biology. These studies will potentially underpin the development of improved diagnostics, therapeutics and novel molecular tools for differentiating species and strains within the Angiostrongylidae. As more data becomes available, it is becoming increasingly apparent that *A. cantonensis* genetically diverse, probably attributable to its sexual reproductive cycle. Sex determination occurs via an XX/XO system, whereby males receive a single X-chromosome and females receive two, resulting in a diploid chromosome number of 11 and 12, for males and females, respectively (Eamsobhana *et al*. 2009). Genetically distinct strains of *A. cantonensis* can possess significant differences in infectivity, fecundity and virulence (Lee *et al*. 2014).

Certain *Angiostrongylus* spp. mitochondrial genes are recognized as a source of genetic diversity. Nine unique *COI* gene haplotypes were identified in a study of Brazilian *A. cantonensis* isolates (Monte *et al*. 2012). Wang and Lv (2014) identified 39 *COI* haplotypes in isolates from China. In studies of Thai isolates, as many as 15 mitochondrial *cytochrome b* gene haplotypes were identified (Dusitsittipon *et al*. 2015; Yong *et al*. 2015*b*). By comparison, only six 18S rDNA haplotypes are known (Eamsobhana *et al*. 2013*a*, 2015*a*).

Nuclear genomes

The *A. cantonensis* nuclear genome is large compared with other nematodes (Zarowiecki and Berriman, 2015). Two *A. cantonensis* nuclear genomes have been sequenced; one (~253 Mb) from a Taiwanese isolate (Morassutti *et al*. 2013*b*) (GenBank accession: PRJEB493) and the other (~260 Mb) from a Thai isolate (Yong *et al*. 2015*a*) (GenBank accession: PRJNA260338). Morassutti *et al*. (2013*b*) detected 28 080 putative open reading frames, 3370 of which possessed homology to protein sequences in public databases. Yong *et al*. (2015*a*) detected 17 482 genes greater than 300 base pairs, 7737 of which were predicted to encode proteins. Of the proteins with known function, most were kinases and transferases. Genes encoding cellular components, including components of the cytoskeleton, were the most abundant (Yong *et al*. 2015*a*). While most genes obtained matches *Caenorhabditis* spp. sequences, *A. cantonensis* shares a closer phylogenetic relationship to other bursate nemotodes such as *Haemonchus contortus* and *Necator americanus* (Morassutti *et al*. 2013*b*; Yong *et al*. 2015*a*).

Morassutti *et al*. (2013*b*) identified genes encoding immunogenic proteins using previously published mass spectrometry data to interrogate their genome. A cDNA walking strategy was used to obtain the complete coding sequence for these genes, with the intention of generating recombinants that might facilitate diagnostic development. Of the immunogenic proteins of interest, six galectins were identified as candidates for expression (Morassutti *et al*. 2012*b*). Yong *et al*. (2015*a*) noted nine galectins amongst 34 excreted-secreted proteins identified in their *A. cantonensis* genome.

Wolbachia spp. are endosymbionts of some arthropods and certain filarial nematodes including *Brugia, Wuchereria* and *Onchocerca* spp. (Ferri *et al*. 2011; Slatko *et al*. 2014). In *Brugia malayi* the *Wolbachia* endosymbiont is essential for survival and fertility (Foster *et al*. 2005). Tsai *et al*. (2007*b*) provided PCR evidence for a *Wolbachia* endosymbiont in *A. cantonensis* which was unusual, given that

Wolbachia had never been reported in a non-filarial nematode (Foster *et al*. 2008). To confirm this, Foster *et al*. (2008) used PCR and immunohistochemistry to examine *A. cantonensis* and *A. costaricensis* but found no evidence of *Wolbachia*. In support of this, Morassutti *et al*. (2013*b*) and Yong *et al*. (2015*a*) report no evidence of *Wolbachia* in their genome sequences. While Foster *et al*. (2008) suggest that the findings of Tsai *et al*. (2007*b*) may be the result of contamination, it should be noted that not all specimens within a filarial nematode species harbour *Wolbachia* even if that species is known to possess a *Wolbachia* endosymbiont (Ferri *et al*. 2011).

Mitochondrial genomes

The mitochondrial (mt) genomes of *A. cantonensis*, *A. mackerrasae*, *A. costaricensis* and *A. vasorum* are similar in size and nucleotide composition. Each circular mt genome encodes 36 genes; 12 proteins, 22 transfer RNAs and two rRNAs (Gasser *et al*. 2012; Lv *et al*. 2012, 2014; Aghazadeh *et al*. 2015*c*; Yong *et al*. 2015*c*). The first mt genomes of *A. cantonensis* and *A. costaricensis* to be sequenced were 13 497 and 13 585 bp in length, respectively, and were 81·6% similar at the nucleotide level (Lv *et al*. 2012). The mt genome of the canine pathogen, *A. vasorum* is 13 422 bp (Gasser *et al*. 2012), and that of *A. mackerrasae* is 13 640 bp (Aghazadeh *et al*. 2015*c*). Pairwise comparisons of translated mt protein coding genes from *A. mackerrasae* to those of *A. cantonensis* revealed only a 2·4% difference, indicating that these species diverged very recently (Aghazadeh *et al*. 2015*c*). This is in contrast to a 16·8% difference observed between *A. mackerrasae* and *A. costaricensis* (Aghazadeh *et al*. 2015*c*).

Lv *et al*. (2014) sequenced mt genomes from seven Chinese isolates of *A. cantonensis* which revealed five unique types. These Chinese mt genomes were compared with that of a Taiwanese and Thai isolate (Yong *et al*. 2015*c*). The Taiwanese and Chinese isolates possess smaller mt genomes than the Thai isolate (Yong *et al*. 2015*c*), which also possessed a genome that was more distantly related compared with the Chinese/Taiwanese genomes. Eighteen tRNAs encoded on the Thai mt genome lacked a TΨC-arm (Yong *et al*. 2015*c*); a tRNA structure that interacts with elongation factor Tu during protein synthesis (Watanabe *et al*. 2014). While the absence of a TΨC-arm is not uncommon in tRNAs encoded on nematode mt genomes (Watanabe *et al*. 2014), differences were observed between isolates; 17 and 16 tRNAs lacked the TΨC-arm in the Chinese and Taiwanese mt genomes, respectively (Yong *et al*. 2015*c*). A second 13 652 bp *A. costaricensis* mt genome was sequenced from a Costa Rican isolate (Yong *et al*. 2015*d*) and compared to the original *A. costaricensis* mt genome, derived from a Brazilian parasite (Lv *et al*. 2012). Based on phylogenetic analyses and genetic distances calculated for the mt genes, it was proposed that these parasites should be considered distinct members of a species complex, or sibling species (Yong *et al*. 2015*d*).

Generally, comparisons of mt genes demonstrate greater resolution for differentiating species/strains within the Angiostrongylidae, compared with the nuclear rDNA. These differences could be exploited to develop molecular tools that differentiate *Angiostrongylus* spp., and may facilitate the identification of new, cryptic species (Gasser *et al*. 2012; Yong *et al*. 2015*d*).

Transcriptomes, proteomes and virulence factors

The transition between larval and adult stages of *A. cantonensis* is a complex process requiring stringent regulation of gene expression. Several studies have investigated differences in gene expression at the transcript and protein level between stages, to identify mechanisms associated with stage transition and function in *Angiostrongylus* spp. While some genes are expressed constitutively across all stages, some are stage specific. Chang *et al*. (2014*a*) found that transcripts exclusive to L_5 worms were related to growth, development, sexual differentiation and reproduction, while L3-specific transcripts were mostly related to metabolism. The classes of proteases expressed between these two stages also differed (Chang *et al*. 2014*a*). Huang *et al*. (2013) observed differences in galectin abundance in the soluble proteomes of male L_5 & male adult worms, and female adult & male adult worms. Stage- and sex-dependent differences in expression of aspartic protease and ornithine decarboxylase antizyme were also noted (Hwang *et al*. 2010; Chen *et al*. 2013), as were differences in antioxidant capacity (Song *et al*. 2012; Huang *et al*. 2013).

Transcriptomes sequenced from all worm stages suggest that proteases are fundamental to *Angiostrongylus* spp. (Ansell *et al*. 2013; Wang *et al*. 2013*b*). Proteases are important virulence factors in other pathogenic helminths, essential for tissue penetration and nutrient acquisition (Stack *et al*. 2011; McVeigh *et al*. 2012; Morassutti and Graeff-Teixeira, 2012). Chang *et al*. (2014*a*) identified transcripts for proteases of various types in the L_3 and L_5 stages; most notably metalloproteases, aspartic proteases & cysteine proteases in the L_3 stage and cysteine, aspartic & serine proteases in the L_5 stage. Serine proteases of *A. cantonensis* react to sera from infected patients (Morassutti *et al*. 2012*b*), and a recombinant asparaginyl endopeptidase was recognized by serum from infected BALB/c and ICR strain mice and, by serum from human angiostrongyliasis patients (Chang *et al*. 2014*b*). This supports their role at the host–parasite interface.

High expression of cathepsin B-like proteases 1 and 2 (AC-cathB-1, AC-cathB-2) and haemoglobin-type cysteine protease (AC-haem) occurs in L_3 larvae (Ni *et al*. 2012; Yu *et al*. 2014*a*). As with other helminths, cathepsin B- and D-like proteases of *A. cantonensis* degrade host proteins, including haemoglobin and IgG (Brindley *et al*. 2001; Baig *et al*. 2002; Beckham *et al*. 2006; Jilkova *et al*. 2011; Cheng *et al*. 2012). Expression of AC-cathB-2 is higher in L_3 larvae than in L_1 larvae and adults (Ni *et al*. 2012), while AC-cathB expression is greater in L_4 and L_5 worms (Han *et al*. 2011). High expression of AC-cathBs occurs in the oesophagus of L_3 larvae, supporting their role in host gut penetration (Yu *et al*. 2014*a*; Long *et al*. 2015). In L_4 worms, AC-cathB-1 is excreted/secreted and localizes to the intestine (Cheng *et al*. 2012). These studies indicate that in L_3 larvae, AC-cathBs are probably important for host gut penetration, and in L_4 and L_5 worms, are involved in host tissue destruction during migration.

Metalloprotease I, AC-cathB-1 and AC-cathB-2 were amongst the most abundant transcripts in pepsin-HCl activated L_3 larvae (Chang *et al*. 2011). Excreted/secreted products of L_1 and L_3 larvae possess demonstrable proteolytic activity (Lai *et al*. 2005*b*; Lee and Yen, 2005; Rebello *et al*. 2012). Furthermore, serine protease and metalloprotease inhibitors reduced penetration of L_3 larvae through the intestinal wall of mice (Lee and Yen, 2005). Metalloprotease activity is present in L_1, L_3, L_5 and adult worms (Lai *et al*. 2005*b*), though metalloprotease transcripts were not detected in L_4 worms, an absence that is not understood (He *et al*. 2009). Metalloproteases of *A. cantonensis* also degrade gelatin and host metalloprotease (Lai *et al*. 2005*b*; Adisakwattana *et al*. 2012). In adult *A. vasorum* 4·5% of all functionally annotated transcripts classified as proteases by homology, including cysteine proteinase 3, aspartyl protease and cathBs (Ansell *et al*. 2013). In adult worms, these proteases probably play a role in the digestion of haemoglobin and other host proteins (Cheng *et al*. 2012; Ansell *et al*. 2013). A role for AC-cathB-2 in reproduction is also proposed, given its localization to the vas deferens, uterus and oviduct of adult worms (Yu *et al*. 2014*a*).

Helminth-derived molecules that regulate host immune responses are considered important virulence factors; inducing an immune state that tolerates the parasites' presence. Extracts from *Angiostrongylus* spp. reduced inflammation in a rodent model of asthma (Pitrez *et al*. 2015). Similarly, asthmatic rats treated with *A. cantonensis* cystatin experienced reduced airway inflammation (Ji *et al*. 2015). Transcripts for immunomodulatory proteins were among the most abundant in adult *A. vasorum* (Ansell *et al*. 2013). Homologues of *Ancylostoma* secreted protein (activation-associated secreted proteins or ASPs) were noted in *A. vasorum*. Helminth ASPs modulate host leucocyte activity and recruit neutrophils by neutrophil receptor binding (Ansell *et al*. 2013). Additionally, ASP was identified in a cDNA library of L_4 *A. cantonensis* larvae and a recombinant version of this protein was recognized by sera from infected mice but not from infected rats (Yang *et al*. 2013*b*). The ASP gene was highly transcribed in brain stage L_4 larvae, and immunization of mice with recombinant ASP before infection reduced neutrophil infiltration in mouse brains, implicating ASPs as being directly involved in the pathogenesis of AEM (Yang *et al*. 2013*b*). Ansell *et al*. (2013) also identified homologues of high mobility group box protein in adult *A. vasorum*. Homologues of this protein from filarial nematodes possess an immunomodulatory role, triggering secretion of pro-inflammatory cytokines such as IL-6 and tumour necrosis factor (TNF)-α by mouse peritoneal macrophages (Thirugnanam *et al*. 2012).

Constituents of the cytoskeleton, galectins and heat-shock proteins are among the most abundant proteins expressed by *A. cantonensis*, based on proteomic studies (Leon *et al*. 2007; Song *et al*. 2012; Huang *et al*. 2013; Chen *et al*. 2014*a*). Galectins participate in a diverse range of processes including cell adhesion and host immune modulation (Morassutti and Graeff-Teixeira, 2012; Huang *et al*. 2013). Galectins also react with serum from angiostrongyliasis patients (Morassutti *et al*. 2012*b*). Stage-specific expression of galectin 1 has been noted; its expression is elevated 11-fold in male L_5 worms compared with male adults (Huang *et al*. 2013). Expression of some galectins is also higher in female adult worms compared with males and vice versa (Song *et al*. 2012; Huang *et al*. 2013), though the significance of this is unknown.

Nitric oxide is produced by the host immune system in response to infections with helminths and other pathogens, to subject them to conditions of oxidative stress (Muro and Perez-Arellano, 2010). Consequently, helminths have evolved defences to counter this. Expression of *A. cantonensis* galectin 10 increased following exposure to H_2O_2, suggesting its involvement in the prevention of oxidative stress (Liu *et al*. 2013). Glutathione-S-transferase (GST) is also involved in helminth defences against oxidative stress, by detoxifying harmful compounds produced by oxygen free radicals (Vibanco-Perez and Landa-Piedra, 1998; Kampkotter *et al*. 2003). Consequently, GST has long been considered a potential target for anthelmintic chemotherapy (Vibanco-Perez and Landa-Piedra, 1998). GST family proteins are highly expressed in adult female *A. costaricensis* (Leon *et al*. 2007). Antioxidants such as peroxiredoxin and thioredoxin peroxidase are also highly expressed, and represent potential chemotherapeutic targets (Leon *et al*. 2007; Huang

et al. 2013). Female adult *A. cantonensis* possess increased antioxidant capacity compared with female L_5 worms (Huang *et al*. 2013), suggesting that female worms increase defences against oxidative stress as they mature, and possibly in preparation for embryogenesis.

MicroRNAs

MicroRNAs (miRNA) are small non-coding RNAs (~21–23 nt) that regulate a wide range of biological processes in parasitic helminths, including development, metabolism and cell differentiation/proliferation (Chen *et al*. 2011*a*; Britton *et al*. 2014). Consequently, miRNAs have been investigated for their role in helminth biology and as potential diagnostic biomarkers of helminth infection (Britton *et al*. 2014).

Chen *et al*. (2011*a*) detected 1 072 876 miRNA species in adult *A. cantonensis*; 122 051 were common to both sexes, 548 720 were female specific and 402 105 were male specific. The miRNA profiles differed between young adult and mature adult worms, though certain miRNA species, such as mirR-1, mirR-71 and miR-44, were expressed highly in both stages (Chang *et al*. 2013). Mir-71 family members are the most abundant miRNA species in young adult and mature adult worms (Chen *et al*. 2011*a*; Chang *et al*. 2013). Mir-71 is thought to promote longevity and stress resistance and along with aca-miR-1–1, may also affect sexual differentiation and development (Chang *et al*. 2013). In L_3 larvae, mir-200c was the most abundant miRNA identified, and its expression was 352-fold higher in L_3 larvae compared with L_4 larvae (Li *et al*. 2014*b*). Li *et al*. (2014*b*) noted higher expression of miR-124 in L_4 worms, and implicate it as an immunomodulator. Mouse microglial cells transfected with miR-124 mimics significantly down-regulated transcription of IL-6, IL-1β and TNF-α (Li *et al*. 2014*b*).

The utility of miRNAs as biomarkers for *A. cantonensis* infection is also under investigation. Expression of miR-132-3p in mouse brains, and of aca-miR-146a/aca-miR-146a-5p in rodent sera and brains, is elevated in infected animals compared with uninfected controls (Chen *et al*. 2014*b*; Li *et al*. 2014*b*; Yu *et al*. 2014*b*). Consequently, aca-miR-146a and aca-miR-146a-5p could serve as useful biomarkers for early-stage *A. cantonensis* infection.

TREATMENT

Treatment of angiostrongyliasis involves reducing inflammation, reducing ICP, eradicating the worms and reducing pain. Mild cases often resolve spontaneously without therapy, though rarely, severe cases can lead to permanent neurologic damage or, death in 2–3% of cases (Eamsobhana and Yong, 2009; Morton *et al*. 2013). Repeated lumbar puncture is performed to reduce ICP, thereby relieving severe headaches and other neurological derangements (Jitpimolmard *et al*. 2007; Graeff-Teixeira *et al*. 2009; Diao *et al*. 2011; Murphy and Johnson, 2013). Analgesics such as ibuprofen can also be administered to relieve headache and fever (Wang *et al*. 2010). Administration of intravenous mannitol or glycerol/fructose can reduce ICP in AEM patients (Li *et al*. 2008; Wang *et al*. 2010). Gamma-globulin has also been administered to AEM patients to support immunity (Li *et al*. 2008).

Ocular angiostrongyliasis though non-fatal, can lead to permanent visual impairment if untreated (Sawanyawisuth *et al*. 2007; Feng *et al*. 2013). Surgical removal of larvae from the eye is often performed, along with administration of topical or oral corticosteroids to reduce inflammation (Patikulsila *et al*. 2003; Kumar *et al*. 2005; Sawanyawisuth *et al*. 2007; Feng *et al*. 2013). Laser killing of larvae in the eye can be performed, and is considered preferable to surgery, provided the worms have caused minimal damage at the time of diagnosis (Feng *et al*. 2013). However, some suggest there is little evidence that laser interventions or surgical removal of larvae improve clinical outcomes (Kumar *et al*. 2005; Sawanyawisuth *et al*. 2007). Improvements have been noted upon administration of anthelmintics (Wang *et al*. 2006*b*), though some investigators advise against this given the risk that dead worms may activate serious intraocular inflammation (Feng *et al*. 2013).

Anthelmintics such as albendazole, mebendazole, flubendazole and ivermectin have been administered in many AEM cases, though their usefulness in AEM management is controversial (Murphy and Johnson, 2013). Some studies suggest that early administration of anthelmintics in AEM may exacerbate symptoms and hasten disease progression (Slom *et al*. 2002; Hidelaratchi *et al*. 2005; Liu *et al*. 2006; Wang *et al*. 2006*a*). Transaminitis has also been reported following albendazole treatment in an AEM case (Morton *et al*. 2013). However, a double blind, placebo controlled trial confirmed a reduction in headache duration in AEM patients, with no adverse effects, though with borderline efficacy, using albendazole (Jitpimolmard *et al*. 2007; Murphy and Johnson, 2013). Albendazole alone or in combination with corticosteroids, was efficacious, and caused no adverse reactions in AEM mouse models (Lan *et al*. 2004; Tu and Lai, 2006). Similarly, administration of albendazole and prednisolone to a group of 53 Thai patients with eosinophilic meningitis caused no serious adverse reactions (Chotmongkol *et al*. 2009).

Corticosteroids such as dexamethasone and prednisolone form the basis of AEM treatment, by reducing granulomatous inflammation and headache intensity

(Sawanyawisuth *et al*. 2004; Sawanyawisuth, 2008; Thanaviratananich and Ngamjarus, 2015). The efficacy of corticosteroid & anthelmintic combination therapy for alleviating AEM symptoms is supported by several independent reports (Wan and Weng, 2004; Chotmongkol *et al*. 2006; Leone *et al*. 2007; Diao *et al*. 2009; Lv *et al*. 2009*a*; Zhou *et al*. 2009; Ueda *et al*. 2015). Furthermore, experiments using peripheral blood mononuclear cells extracted from AEM patients indicate that dexamethasone & albendazole combination therapy dampens the Th2 cytokine response in favour of a Th1 response (Diao *et al*. 2009). Generally, corticosteroids are administered in doses ranging from 3 mg day^{-1} to 20 mg four times daily, for 1–2 weeks (Chotmongkol *et al*. 2000, 2006; Li *et al*. 2008; Zhou *et al*. 2009; Wang *et al*. 2010; Murphy and Johnson, 2013). Albendazole or mebendazole are administered at doses of 10–20 mg kg^{-1} day^{-1} for 1–2 weeks (Chotmongkol *et al*. 2006; Zhou *et al*. 2009; Wang *et al*. 2010). However, no randomized, double-blind, placebo-controlled trials have been performed to assess the efficacy of these regimens, and some investigators report little or no benefit following their administration (Ali *et al*. 2008; Hochberg *et al*. 2011; Tseng *et al*. 2011).

Chotmongkol *et al*. (2009) found no statistical difference between two study groups of more than 50 eosinophilic meningitis patients, treated with either albendazole and prednisolone combination therapy or with prednisolone alone. Furthermore, in rodent models of AEM, dexamethasone treatment alone reduced brain cell apoptosis, BBB permeability and expression of the 14-3-3β protein in CSF (Tsai *et al*. 2014*b*, 2015), casting doubt on the therapeutic benefit of administering anthelmintic drugs for the treatment of AEM.

Alternative therapies are also under investigation in rodents. Tribendimidine, a broad spectrum anthelmintic developed in China in the 1980s (Robertson *et al*. 2015), killed *A. cantonensis* in rodents, possibly by damaging its tegument (Wang *et al*. 2013*a*; Zeng *et al*. 2013*a*; Feng *et al*. 2014). Treatment of mice with IL-12 and mebendazole reduces AEM severity and causes a shift towards a Th1 response (Du *et al*. 2003). Lai *et al*. (2005*a*) used albendazole in combination with GM6001 (a matrix MMP-9 inhibitor) to reduce AEM severity in mice. In combination with albendazole, certain Chinese herbs and their extracts exhibited some beneficial effects in mouse models of *Angiostrongylus* optic neuritis and AEM (Lai, 2006; Feng *et al*. 2015). Ginger extracts possess some activity against *A. cantonensis* (Lin *et al*. 2010). Curcumin (a compound found in turmeric) was not efficacious in AEM mouse models when used alone, though in combination with albendazole, reduced CSF eosinophilia to a greater extent than albendazole mono-therapy (Shyu *et al*. 2012). Similarly, the combination of diammonium glycyrrhizinate (a liquorice root extract) and albendazole was more effective in a mouse model of AEM than albendazole/dexamethasone co-therapy (Li *et al*. 2013*b*). The marine fungal extract m2-9 had synergistic effects when used in combination with albendazole for treatment of murine AEM (Li *et al*. 2012). Treatment with albendazole and thalidomide was time-dependent; early treatment produced better outcomes in murine AEM (Chen and Lai, 2007).

To summarize, lumbar puncture is effective at reducing ICP in AEM, relieving patients of the associated headaches (Graeff-Teixeira *et al*. 2009; Murphy and Johnson, 2013). Administration of corticosteroids to reduce inflammation in AEM is also warranted based on multiple reports (Chotmongkol *et al*. 2009; Diao *et al*. 2009; Murphy and Johnson, 2013). Conversely, the value of administering anthelmintics is uncertain (Jitpimolmard *et al*. 2007; Chotmongkol *et al*. 2009; Murphy and Johnson, 2013). As reports of severe adverse events resulting from albendazole use are rare, it should generally be considered safe for AEM treatment, particularly in combination with corticosteroids (Murphy and Johnson, 2013). However, given the borderline efficacy of albendazole monotherapy (Jitpimolmard *et al*. 2007), and the lack of any difference in clinical outcome when comparing corticosteroid monotherapy and corticosteroid/albendazole co-therapy (Chotmongkol *et al*. 2009), the value of administering anthelmintics for AEM treatment is questionable. Given the inconsistencies, these studies represent a useful guide for the treatment of angiostrongyliasis at best. Clearly, further research, including randomized, double-blind, placebo-controlled trials, is required to establish an improved consensus for the treatment of this disease.

PREVENTION AND CONTROL

Given its wide distribution and zoonotic nature, it is unlikely that *A. cantonensis* can be completely eradicated. However, disrupting its transmission to humans through the control of its preferred hosts is plausible. Molluscicides and other methods to control snail and slug numbers have been implemented in Hawaii and China (Hata *et al*. 1997; Hollingsworth *et al*. 2013; Yang *et al*. 2013*a*). In Jamaica, molluscicides are routinely applied to growing produce but this practice was deemed superficial as molluscs remain in the surrounding vegetation and return presumably when the molluscicides lose their potency (Waugh *et al*. 2005). In China, the collection of *P. canaliculata* egg masses and the use of commercial molluscicides have proved effective, though their use is limited by their laborious nature and potential environmental side effects (Yang *et al*. 2013*a*). Biological controls, including the introduction of ducks and *Mylopharyngodon piceus* (black carp) to rice paddy fields and ponds drastically

reduced snail numbers (Yang *et al.* 2013*a*). Hollingsworth *et al.* (2013) suggests drowning of terrestrial slugs or snails for several days in a covered bucket filled with soapy water or a 15% solution of salt water. The salt water treatment is not only lethal to terrestrial molluscs, but is also expected to kill any *A. cantonensis* larvae that may be present (Hollingsworth *et al.* 2013).

Standardized food treatment measures may be effective in some areas, though would be ineffective in locations where food is often home grown or collected from the local environment. Thorough washing of vegetables prior to consumption is a simple measure to reduce *A. cantonensis* transmission (Cowie, 2013*b*). Washing experiments carried out on grown produce by Yeung *et al.* (2013) showed no difference between simple water washes and washing with other domestic chemicals such as bleach, acetic acid and sodium chloride. While some slug slime residue remained after washes, washing was still considered the most appropriate measure for reducing the transmission of larvae in produce (Yeung *et al.* 2013). As evidenced by Wang *et al.* (2011), thorough roasting of molluscs for more than 20 min is required to ensure that all *A. cantonensis* larvae are destroyed in mollusc tissue.

The major difficulty in controlling the spread of angiostrongyliasis lies in its link with diverse social, economic, cultural and environmental factors. In some endemic areas, awareness of *A. cantonensis* is low in the general community (Li *et al.* 2011). Consequently, educating the public to the dangers of raw mollusc consumption in these regions may be an effective starting measure. Educating laypeople on strategies for controlling the spread of *A. cantonensis* would be helpful, particularly in regions where food is home grown or collected locally. However, public education programmes must be executed with caution, as the public concern generated could be excessive given the rarity of angiostrongyliasis. Despite this, public education campaigns have had favourable results in China (Lv *et al.* 2008). Finally, programmes that raise awareness amongst public health workers and physicians might enable them to provide better advice to patients planning overseas travel, and facilitate a more rapid diagnosis with improved clinical outcomes when future cases of angiostrongyliasis are encountered.

Concluding remarks

While angiostrongyliasis is generally considered rare, its global presence is steadily increasing with both clinical cases and epidemiological surveys confirming *A. cantonensis* in regions where it was previously considered absent. AEM is often overlooked at the outset, particularly in regions where angiostrongyliasis is rarely observed. There is a lack of standardization in the procedures for diagnosing angiostrongyliasis. Serological tests are commercially available, though are not widely used. Other diagnostic tools are under development. There is no consensus on the appropriate treatment for angiostrongyliasis and some treatments are controversial. A combination of anthelmintics and corticosteroids seems effective in most cases, though there have been no randomized, double-blind, placebo-controlled trials to support any treatment regimen described for angiostrongyliasis. Blocking transmission is the most appropriate method of reducing infections. Implementing simple wash protocols for vegetables, public education on the dangers of raw mollusc consumption and implementation of mollusc and rat control measures may be useful. In developed nations, the prevalence of angiostrongyliasis in companion animals and wildlife is usually greater than in humans. Thus, animals play a role in alerting public health authorities of the risk to humans in locations where animals are affected by angiostrongyliasis. Considering the potentially lethal nature of angiostrongyliasis and the recent reports of its increasing geographical range, it is important that this disease is given due consideration in the differential diagnosis of eosinophilic meningitis, even in areas where *A. cantonensis* is rarely reported. This is essential to improve patient prognosis and reduce human and companion animal suffering.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S0031182016000652.

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