An epidemiological study of *A. cantonensis* in Jamaica subsequent to an outbreak of human cases of eosinophilic meningitis in 2000

C. A. WAUGH[1], J. F. LINDO[2], J. LORENZO-MORALES[3] *and* R. D. ROBINSON[1]*

[1] *Department of Life Sciences, The University of the West Indies, Mona, Kingston 7, Jamaica*
[2] *Department of Microbiology, The University of the West Indies, Mona, Kingston 7, Jamaica*
[3] *Institute of Tropical Diseases and Public Health of the Canary Islands, The University of La Laguna, Tenerife, Canary Islands*

SUMMARY

The infection status of angiostrongylosis in Jamaica was assessed in wild rats and molluscs in the 5 years following the major outbreak of eosinophilic meningitis (EM) in 2000. Parasitological analyses of 297 *Rattus rattus* and 140 *Rattus norvegicus*, and 777 terrestrial molluscs from all 14 Parishes on the island revealed *Angiostrongylus cantonensis* in 32·0% of the rats and in 12·5% of the molluscs. Multivariate analyses confirmed that *A. cantonensis* occurred significantly more frequently in *R. rattus* (Odds Ratio [OR] = 1·76), while mean infection intensity in *R. rattus* was also significantly higher (16·8) than *R. norvegicus* (11·3) (Mann–Whitney *U*-test: $P = 0·01$). Third-stage larvae of *A. cantonensis* were detected in 29% of 86 *Pleurodonte* spp.; in 20% of five *Poteria* spp.; in 18·7% of 369 *Thelidomus asper*; in 11% of 18 *Sagda* spp.; and in 6% of 24 veronicellid slugs. Most rodent infections occurred in Northeastern Jamaica (OR = 11·66), a region where infected molluscs were also abundant. Given the prevalence of *A. cantonensis* infection in rats has significantly increased since the 2000 outbreak, and that a survey of human infections revealed at least ten autochthonous cases in the last 15 years, angiostrongylosis persists as an important zoonosis in Jamaica.

Key words: *Angiostrongylus cantonensis*, Rat lungworm, *Rattus*, *Pleurodonte*, *Thelidomus*, eosinophilic meningitis, emerging infectious disease, Jamaica.

INTRODUCTION

The 'Rat Lungworm' *Angiostrongylus cantonensis* (Nematoda: Metastrongylidae) was first described from the lungs of captive rats in Southern China (Chen, 1935) and is the parasite most commonly causing human eosinophilic meningitis (EM) in many parts of the world (Qvarnstrom *et al.* 2013). In 1994, a case of autochthonous EM was described in an adult Jamaican who had had not travelled outside the country (Barrow *et al.* 1996) and, although confirmatory histology or serology was not conducted, the finding raised important questions regarding the endemic status of *A. cantonensis* in the island. Four years later, the autopsy report of a 14-month-old boy revealed adult *A. cantonensis* in his lungs and larvae in the meninges (Lindo *et al.* 2004) and became the first confirmed case of human infection with the parasite in Jamaica. Also in 2000, 12 of 23 vacationing US medical students in Jamaica, who earlier had shared a salad meal at a hotel, developed EM within weeks of returning to the USA (Slom *et al.* 2002; Murphy and Johnson, 2013). This outbreak is considered the largest for EM in the Western world (Slom *et al.* 2002). The

endemic status of *A. cantonensis* in Jamaica was ultimately confirmed by Lindo *et al.* (2002) who revealed that 22% of 109 wild rats were infected with *A. cantonensis* and that four of ten *Thelidomus asper*, a commonly occurring land snail in Jamaica, harboured third-stage larvae of the parasite. Elsewhere in the Caribbean, five cases of human EM, presumed to be caused by *A. cantonensis*, were reported from localities in or near Havana, Cuba (Pascual *et al.* 1981), while De Meuron (2005) has described four paediatric cases from Martinique.

Adult *A. cantonensis* occurs naturally in the pulmonary arteries and right ventricle of numerous wild rodents, including *Rattus* spp. (Spratt, 2015). Briefly, first-stage larval worms are expelled in the feces and enter a wide range of terrestrial molluscs through ingestion or penetration. Following consumption of infected snails or slugs, third-stage larvae penetrate the intestine of rodents and enter the blood stream. A portion of this population invades the central nervous system where high-intensity infections are associated with neuropathology. After a week or so, fourth-stage larvae re-enter the blood from the brain and establish as sexually mature adults in the pulmonary arteries. Eggs are carried to the lung capillaries where they hatch and larvae penetrate the respiratory tissues. These are

* Corresponding author: Department of Life Sciences, The University of the West Indies, Mona, Kingston 7, Jamaica. E-mail: ralph.robinson@uwimona.edu.jm

then carried up the trachea, are swallowed, and are eventually released in feces about 6 weeks post-infection (Mackerras and Sandars, 1955). It is also possible that infections are transmitted by paratenic hosts, which include freshwater shrimps, land crabs, predatory planarians, amphibians and reptiles (see Thiengo *et al*. 2013). Humans are exposed to *A. cantonensis* infection by ingestion of third-stage larvae in infected snails or slugs either accidentally, as food, or even as a dare to consume raw snail tissue (see Cowie, 2013*a*, *b*). Infection through ingestion of raw vegetables contaminated with snail slime has also been suggested (Waugh *et al*. 2005; Giannelli *et al*. 2015).

In the Caribbean, *A. cantonensis* has been recovered in rats from Cuba, Dominican Republic, Grenada, Haiti, Martinique, Puerto Rico and Jamaica (see Robinson *et al*. 2013). The parasite was introduced to mainland USA in the mid-1980s, probably by infected rats from ships docking in New Orleans (Campbell and Little, 1988; Louisiana Office of Public Health, 2006). Currently, *A. cantonensis* is generally restricted to the tropics and subtropics. However, it has been suggested that the natural range of the parasite will expand because of climate change (Zhang *et al*. 2008; Lv *et al*. 2011). On the other hand, York *et al*. (2014) contended that climate change may actually reduce the total geographic area of most suitable climatic conditions for *A. cantonensis* during the coming decades. In either event, the parasite is likely to continue to expand its current range in the near future due to introductions and host expansion.

The paper reports baseline data on angiostrongylosis following the 2000 emergence in Jamaica, against which future surveys aiming to track emergence may be compared. Specifically, the island-wide geographic distribution and levels of infection of *A. cantonensis* in wild rats and terrestrial molluscs in Jamaica are reported together with a brief review of human cases to date. The paper also includes recommendations for required research.

MATERIALS AND METHODS

Wild rat data

During the period 2002–2005, 437 wild rats (*Rattus rattus* and *Rattus norvegicus*) were captured in Jamaica using baited (smoked red herring) snap traps, glue traps, poison (warfarin) traps and live traps; road-kill was also accepted. The rats were collected from all 14 Parishes (Kingston and St. Andrew were merged) comprising the four Regional Health Authorities (RHA) on the island. Health-management personnel assigned to a RHA are responsible for the provision of health care for the population living within its boundaries and report to the Ministry of Health.

Post-capture, the rats were tagged for geographic location, bagged and returned on ice for analysis. Morphometric data on the rats [sex, weight and snout-to-vent length (SVL)] were collected, and the species confirmed. A measure of physical condition of each rat was generated as an independent variable from Studentized residual values from least-squares regression of $\log_{10}$ weight (g) *vs* $\log_{10}$ SVL (cm) (after Wilson, 1991).

Angiostrongylus *in wild rats*

Adult *A. cantonensis* were recovered from the right ventricle and pulmonary arteries of infected rats using fine forceps and a stereomicroscope while taking care not to break the worms. They were then counted, fixed in 10% formalin–glycerol and stored in 70% ethanol. Confirmation of species was based on Commonwealth Institute of Helminthology Keys (Anderson *et al*. 2009). Voucher Specimens of *A. cantonensis* were deposited as Zoological Accessions [#]2906 in the Department of Life Sciences, University of the West Indies, Kingston.

Descriptive terms of parasite prevalence and mean intensity adhere to Margolis *et al*. (1982) with revisions by Bush *et al*. (1997). Univariate associations between host variables (species, sex, $\log_{10}$ weight (g), $\log_{10}$ SVL (cm), physical condition and geographic location at capture) and parasite variables (prevalence and intensity) were sought using Quantitative Parasitology v3.0 (Rózsa *et al*. 2000; Reiczigel, 2003) and Statistical Package for the Social Sciences (SPSS) v12 for Windows®. Exact confidence limits for the population prevalence of *A. cantonensis* in wild rats were based on Sterne's exact method (see Reiczigel, 2003). Binary logistic regression was used to model prevalence of *A. cantonensis* using variables achieving $P < 0.20$ in the univariate analyses. Risk factors for infection were expressed as odds ratios (OR) and 95% confidence intervals (CI).

Angiostrongylus *in terrestrial molluscs*

To determine the terrestrial molluscs most likely to be involved in transmission of *A. cantonensis* in Jamaica, collections were made from 30 locations spanning the four RHA that were known to harbour infected rats. In total, 777 adult terrestrial snails and slugs were gathered from the surface of the soil, under logs, leaves, etc. The molluscs were then placed in individual plastic bags pending documentation and parasitological analysis. Identification of the snails was based on morphology, reference to Voucher Specimens held at the Institute of Jamaica, Kingston, and interactive keys by Rosenberg and Drumm (2004).

Parasitological investigation of the snails and slugs involved excision of the cephalopodal mass followed

by incubation at 37 °C in an aqueous solution of 0·2% pepsin and 0·7% HCl for 4 h (after Wallace and Rosen, 1966). Larvae of *Aelurostrongylus abstrusus* (Railliet), a lungworm usually of cats, were occasionally encountered in the snails, but these were readily distinguished from *A. cantonensis* (see Ash, 1970). Briefly, third-stage larvae of *A. abstrusus* tended to be longer than *A. cantonensis* (mean 520 μM compared with 475 μM); have a longer oesophagus (mean 210 μM compared with 180 μM); and possessed rounded rather than fine-pointed tail terminations.

RESULTS

Wild rat data

In total, 437 wild rats (297 *R. rattus* and 140 *R. norvegicus*) were collected from 14 Parishes comprising the four RHAs in Jamaica. *Rattus norvegicus* was significantly longer than *R. rattus* (means = 16·98 and 14·67 cm, respectively; $F = 40.01$; $P < 0.001$) and heavier (means = 205·4 and 118·8 g respectively; $F = 82.57$; $P < 0.001$). Based on residual values (length-corrected weight) accruing from the regression of $\log_{10}$ weight *vs* $\log_{10}$ SVL, *R. norvegicus* appeared to be in better physical condition than *R. rattus* ($F = 31.97$; $P < 0.001$). Physical condition, however, did not differ between the sexes within either species.

Angiostrongylus *in wild rats*

Adult *A. cantonensis* males measuring 15–20 mm and females measuring 18–28 mm (based on measurement of at least ten worms from rats trapped in each of the Parishes) were recovered from the right ventricle and pulmonary arteries of 32·0% (95% CI = 28–37%) of the rats. The prevalence and mean intensity of *A. cantonensis* infection in *R. rattus* and *R. norvegicus* is shown in Table 1. *Rattus rattus* was significantly more likely to be infected with *A. cantonensis* (35·4% of 297 rats) than *R. norvegicus* (25·0% of 140 rats) ($\chi^2 = 4.684$; $P = 0.037$) and harboured significantly more worms (mean = 16·8) than *R. norvegicus* (mean = 11·3) (Mann–Whitney *U*-test: $P = 0.01$). Infection intensity ranged 1–76 worms. Although strong associations between both the prevalence ($F = 17.102$; $P < 0.001$ and $F = 17.001$; $P < 0.001$ for *R. rattus* and *R. norvegicus*, respectively) and intensity ($F = 4.927$; $P = 0.027$ and $F = 9.187$; $P = 0.003$ for *R. rattus* and *R. norvegicus*, respectively) were observed with $\log_{10}$ SVL, none was observed with either $\log_{10}$ weight or body condition in either species of rats. Further, comparisons of the prevalence or mean intensity of *A. cantonensis* between the host sexes revealed no significant associations. On the other hand, the parasites were aggregated in both species of rats (index of discrepancy, $D > 0.8$) (Poulin, 1993).

Table 1. Prevalence and mean intensity of infection with *Angiostrongylus cantonensis* in *Rattus rattus* and *R. norvegicus* in Jamaica

Rat	Prevalence (*n*; 95% CI)[a]	Mean intensity (95% CI)
R. rattus	35·4% (297; 29·6–40·7)	16·8 (14·19–19·7)
R. norvegicus	25·0% (140; 18·7–33·8)	11·3 (8.31–15·9)

[a] Sterne's exact method (Reiczigel, 2003).

The prevalence of *A. cantonensis* differed significantly in rats trapped in the different RHAs ($\chi^2 = 48.319$; $P < 0.001$). Fifty one per cent of 151 rats emanating from the north-eastern RHA were infected, followed by rats from the southern, south-eastern and western regions (Table 2). On a Parish level, *A. cantonensis* infections ranged from 4% of 27 rats from Trelawny (western RHA) to 60% of 20 rats from St. Mary (north-eastern RHA). No significant associations were detected between the mean intensities of *A. cantonensis* in infected rats and geographic location.

Multivariate analyses were performed to determine significant independent host factors that achieved $P < 0.02$ in the univariate analyses (i.e. species and $\log_{10}$ SVL) and geographical predictors (RHA) of the distribution of the parasites. A binary logistic regression model confirmed the statistically significant univariate analyses that *R. rattus* was more likely to be infected with *A. cantonensis* than *R. norvegicus* (OR = 1·76) and that *A. cantonensis* occurred more frequently in *R. rattus* in the northeastern RHA (OR = 11·66).

Angiostrongylus *in terrestrial molluscs*

Of the 777 snails and slugs examined, 12·5% harboured third-stage larvae of *A. cantonensis*. Infected molluscs included *Pleurodonte* spp. (29% of 86 snails), *Poteria* spp. (20% of 5 snails); *T. aspera* (18·7% of 369 snails), *Sagda* spp. (11% of 18 snails) and veronicellid slugs (6% of 34 individuals). Eighty-two of the 86 specimens of *Pleurodonte* spp. collected were gathered in the north and south-eastern RHAs where infection levels were 10·3% of 29 snails and 41·5% of 53 snails, respectively. In comparison, prevalence rates of *A. cantonensis* in *T. aspera* were not associated with geographical location (RHA).

DISCUSSION

In this survey of 437 wild rats conducted during the 5 years immediately following the 2000 outbreak of angiostrongylosis in Jamaica, we report an overall prevalence of infection of 32% rats. This represents a significantly higher prevalence of infection with

Table 2. *Distribution of Angiostrongylus cantonensis in wild rats in Parishes and Regional Health Authorities (RHA) in Jamaica*

RHA (sample size)	Parish	Number	Parish % prevalence	RHA % prevalence
Southeastern $n = 138$	Kingston and St. Andrew	68	21	26·1
	St. Catherine	40	45	
	St Thomas	30	13	
Northeastern $n = 151$	Portland	76	51	51·0
	St. Mary	20	60	
	St Ann	55	47	
Western $n = 72$	Trelawny	27	4	6·9
	Hanover	18	11	
	St. James	17	6	
	Westmoreland	10	10	
Southern $n = 76$	Manchester	30	57	28·9
	St. Elizabeth	23	13	
	Clarendon	23	9	

A. cantonensis ($\chi^2 = 4\cdot167$; $P = 0\cdot04$) than that reported by Lindo *et al.* in 2002 (22% of 109 rats). Despite the lower sample size in the initial report which could negatively influence prevalence estimates (Jovani and Tella, 2006), it appears that *A. cantonensis* has further established in the rat population during the study period. Further, Lindo *et al.* (2002) reported no significant difference in the rate of infection of *R. rattus* and *R. norvegicus* but we found a significantly higher prevalence and mean intensity of infection in *R. rattus*, establishing this species as an important reservoir of infection in Jamaica.

Infection rates of *A. cantonensis* in rats elsewhere in the Caribbean range 16–100% (Aguiar *et al.* 1981; Andersen *et al.* 1986; Vargas *et al.* 1992; Raccurt *et al.* 2003; Chikweto *et al.* 2009). However, Qvarnstrom *et al.* (2013) recently showed that dissections of rodents, such as conducted in this and other studies, may significantly underestimate the prevalence rates of *A. cantonensis* in definitive hosts in endemic areas: These authors revealed that although 54% of 37 rats in Hawai'i were positive based on morphology, 100% of tissue samples from the same animals were positive using a real-time polymerase chain reaction assay.

More than twice as many *R. rattus* ($n = 297$) than *R. norvegicus* ($n = 140$) were trapped in the present study. This may reflect a higher population density of *R. rattus* in Jamaica, although *R. norvegicus* is dominant in Europe and North America (Burton and Burton, 2002). There appears to be no definitive data on the comparative trappability of *R. norvegicus* and *R. rattus*. However, Webster *et al.* (1994) showed that *R. norvegicus* infected with *Toxoplasma gondii* exhibited behavioural changes that rendered *Toxoplasma*-infected individuals more susceptible to predation, or trapping and poisoning during control programmes.

Aggregated distributions of *A. cantonensis* were encountered in both species of rats in this study, indicating heterogeneity of host behaviour and/or differences in host susceptibility to infection, exposure, genetic factors or immunity (Anderson and Gordon, 1982). Although this also suggests that the parasite (in large numbers) may be detrimental to its host, we did not detect any association between prevalence of infection or worm burden and physical condition in either species of rats. On the other hand, a study of the same 437 rats in Jamaica (Waugh *et al.* 2006) also indicated a significantly higher prevalence of gastrointestinal helminths (35%) in *R. rattus* compared with *R. norvegicus* (18·6%).

The persistence of *A. cantonensis* in the Caribbean and its potential spread throughout the islands and Southern USA has been linked not only to the spread of infected rats but also the abundance of suitable intermediate (Lindo *et al.* 2011) and, possibly, paratenic hosts for the parasite. Of 777 terrestrial snails and slugs examined in this study, 12·5% harboured *A. cantonensis* based on dissection/digestion. Given the ability of *A. cantonensis* to infect a wide range of molluscs in the Caribbean (see Robinson *et al.* 2013) research is urgently needed to assess the intermediate host range of the parasite in Jamaica where more than 560 valid species of terrestrial molluscs have been recognized (Rosenberg and Muratov, 2006). The importance of eating eat raw or undercooked freshwater and terrestrial snails and slugs, thus providing opportunities for human infection with *A. cantonensis*, was highlighted by Zhang *et al.* (2008) in China. However, the potential for direct transmission by land snails can only be assessed with a full understanding of the intermediate host range of the parasite in which case molecular-based studies (see Aziz *et al.* 2016) should focus on common snail species which are present in domestic and farming environments.

Although Alicata (1969) speculated that the giant African land snail, *Achatina fulica*, was important to the spread of *A. cantonensis* to new localities,

Table 3. Reported cases of eosinophilic meningitis (EM) and infections with *Angiostrongylus cantonensis* in Jamaica

Year	Case	Outcome	References
1994	First case of EM reported in Jamaica in a 31-year-old female, St. Ann.	Recovered	Barrow *et al.* (1996)
2000	A 14-month-old boy, Kingston	Fatal	Lindo *et al.* (2004)
	Outbreak in Montego Bay: 12 cases: nine hospitalized/two serious	Recovered with sequelae	Slom *et al.* (2002)
2001	American tourist contracts EM in Jamaica	Recovered	King (2001)
2003	A 27-year-old male prisoner, St. Elizabeth	Fatal	T. Ferguson, personal communication
2004	A 19-month-old female, from St. Catherine, diagnosed in Kingston	Permanent neurological damage	Evans-Gilbert *et al.* (2014)
2004– 2009	A 24-month-old male diagnosed in Kingston	Recovered	Evans-Gilbert *et al.* (2014)
	A 19-month-old male diagnosed in Kingston	Recovered	
	A 12-month-old female diagnosed in Kingston	Hemiparesis	
	An 8-year-old male diagnosed in Kingston	Recovered	
2007	An 8-year-old male diagnosed Kingston	Recovered	Evans-Gilbert *et al.* (2014)
2009	A 30-year-old female; ocular angiostrongylosis; diagnosed in Kingston	Recovered	Mattis *et al.* (2009)
2013	A 19-month-old infant diagnosed in Kingston	Permanent neurological damage	Evans-Gilbert *et al.* (2014)

areas exist such as Jamaica where the parasite is endemic yet *A. fulica* is absent. In the absence of giant African land snails from Jamaica, *A. cantonensis* probably reached here in infected rats from cargo ships as appears to have been the situation in Southern USA (Louisiana Office of Public Health, 2006). It therefore appears that infected rats may be more critical to the expansion of the parasite's geographic range than introduced infected molluscs. Certainly, *A. fulica* appears to have played little part in the establishment of *A. cantonensis* in the Caribbean, but the existence of this huge snail in many islands of the Lesser Antilles may further consolidate infections.

It has not been established whether the mucus slime trail from infected molluscs can transfer infective, third-stage larvae to humans if ingested. Unpublished, preliminary observations by the authors of larval shedding by *A. cantonensis*-infected snails showed that two of 15 infected *T. aspera* released just three third-stage larvae in mucus over a period of 10–15 min, and then only after feeding on fresh lettuce leaves, and that none of 11 infected *Pleurodonte* spp. released larvae of *A. cantonensis* under the same conditions. This apparent low rate of larval release in snail slime is inconsistent with the assumption that contaminated raw vegetables were implicated in the 2000 outbreak of EM on the island (Slom *et al.* 2002). In comparison, Heyneman and Lim (1967) reported third-stage larvae of *A. cantonensis* larvae were shed in the mucus of the Malayan slug *Microparmarion malayanus*, but there are conflicting reports of slime from infected molluscs containing few or no larvae, even when the same animals had high-intensity infections (Qvarnstrom *et al.* 2013). As a priority, studies are

needed to clarify the role of mollusc slime in human disease transmission.

The role of paratenic hosts in the transmission of *A. cantonensis* also requires further study in situations like Jamaica where blended raw molluscs or crustaceans might be included as ingredients in drinks and tonics which form part of the traditional 'grass roots' culture (Waugh *et al.* 2005). One confirmed paratenic host, the American Bullfrog *Rana catesbeiana* (Asato *et al.* 2004) has been present in Jamaica and Puerto Rico for several decades (Mahon and Aiken, 1977) and awaits urgent investigation for *A. cantonensis*. Another, the predatory flatworm *Platydemus manokwari*, although not yet reported from Jamaica, has recently been detected in Puerto Rico and Florida (Justine *et al.* 2015).

The incidence of *A. cantonensis* in endemic areas and its geographical range appear to be increasing worldwide (see Cowie *et al.* 2012). The parasite readily establishes itself in faunas where a large range of mollusc species can act as intermediate hosts and rats are ubiquitous. The wide distribution of *A. cantonensis* in rats across Jamaica suggests that the parasite has been here for some time. Infection prevalence in rats was significantly associated with geographic location with highest prevalence, representing almost two-thirds of all the infected rats in our study, occurring in the northeastern RHA. In contrast, the lowest prevalence of infection occurred in the western RHA. A possible explanation for this is that northeastern RNA boasts the highest forest coverage, rainfall levels (in excess of 300 mm per year) and minimal land utilization compared with the other RHAs in Jamaica. The northeastern RHA is also likely to support a larger population of

terrestrial molluscs – the highest prevalence rate of *A. cantonensis* in *Pleurodonte* spp. occurred in eastern Jamaica – and would be expected to result in increased rat–mollusc interaction. Environmental parameters linked to the transmission of *A. cantonensis* require further investigation (Cowie *et al.* 2012).

As a follow-up to survey of rats and terrestrial molluscs, the medical and scientific communities attached to our major hospitals were contacted regarding to the signs and symptoms of angiostrongylosis and invited to submit reports of suspicious findings for the period 2000–2015. While acknowledging that improved awareness and effort would be expected lead to increased reporting of cases, Table 3 shows, as a matter of record, that about 2 dozen cases of EM or confirmed angiostrongylosis were recorded in Jamaica since 1994, 25% of which were children 2 years old or younger at time of diagnosis. Pica disorder is more commonly seen in women and children in Jamaica (Robinson *et al.* 1990) and in areas of low socioeconomic status (Wong *et al.* 1991) and could be implicated in this apparent bias in infection risk.

Based on this 2002–2005 survey it appears that *A. cantonensis* has further established in the wild rat population in Jamaica since 2000; the study also confirms that several endemic snails can harbour infections at high prevalence. These data, combined with continuing reports (at least ten cases) during the last 15 years of infections in humans who never travelled off-island, indicates that *A. cantonensis* persists as an important zoonosis here. However, we are still uncertain how the parasite is being transmitted but suspect accidental ingestion of small, raw snails (Cowie, 2013*b*) and vegetables contaminated with larvae (Slom *et al.* 2002; Waugh *et al.* 2005), and the role of paratenic hosts in transmission of the parasite in Jamaica still awaits investigation. Meanwhile, the expansion of *A. cantonensis* in Jamaica and should be closely monitored.

REFERENCES

Aguiar, P. H., Morera, P. and Pascual, J. (1981). First record of *Angiostrongylus cantonensis* in Cuba. *American Journal of Tropical Medicine and Hygiene* **30**, 963–965.

Alicata, J. E. (1969). Present status of *Angiostrongylus cantonensis* infection in man and animals in the tropics. *Journal of Tropical Medicine and Hygiene* **72**, 53–63.

Andersen, E., Gubler, D. J., Sorenson, K., Beddard, J. and Ash, L. R. (1986). First report of *Angiostrongylus cantonensis* in Puerto Rico. *American Journal of Tropical Medicine and Hygiene* **35**, 319–322.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (2009). *Keys to the Nematode Parasites of Vertebrates*. p. 480 Commonwealth Agricultural Bureaux International, Wallingford, UK. ISBN-13: 978-1-84593-572-6.

Anderson, R. M. and Gordon, D. M. (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373–398.

Asato, R., Taira, K., Nakamura, M., Kudaka, J., Itokazu, K. and Kawanaka, M. (2004). Changing epidemiology of *Angiostrongyliasis cantonensis* in Okinawa Prefecture, Japan. *Japanese Journal of Infectious Diseases* **57**, 184–186.

Ash, L. A. (1970). Diagnostic morphology of the third-stage larvae of *Angiostrongylus cantonensis, Angiostrongylus vasorum, Aelurostrongylus abstrusus*, and *Anafilaroides rostratus* (Nematoda: Metastrongyloidea). *Journal of Parasitology* **56**, 249–253.

Aziz, N. A. A., Daly, E., Allen, S., Rowson, B., Greig, C., Forman, D. and Morgan, E. R. (2016). Distribution of *Angiostrongylus vasorum* and its gastropod intermediate hosts along the rural–urban gradient in two cities in the United Kingdom, using real time PCR. *Parasites and Vectors*. 9, 56. doi: 10.1186/s13071-016-1338-3.

Barrow, K. O., St. Rose, A. and Lindo, J. F. (1996). Eosinophilic meningitis: is *Angiostrongylus cantonensis* endemic in Jamaica? *West Indian Medical Journal* **45**, 70–71.

Burton, M. and Burton, R. (2002). *International Wildlife Encyclopedia*, 3rd Edn. Marshall Cavendish, New York.

Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**, 575–583.

Campbell, B. G. and Little, M. D. (1988). The finding of *Angiostrongylus cantonensis* in rats in New Orleans. *American Journal of Tropical Medicine and Hygiene* **38**, 568–573.

Chen, H. T. (1935). Un nouveau nematode pulmonaire: *Pulmomema cantonensis* n.g.n.spp. de rats de Canton. *Annales de Parasitologie Humaine et Comparée* **13**, 312–317.

Chikweto, A., Bhaiyat, M. I., Macpherson, C. N. L., De Allie, C., Pinckney, R. D., Richards, C. and Sharma, R. N. (2009). Existence of *Angiostrongylus cantonensis* in rats (*Rattus norvegicus*) in Grenada, West Indies. *Veterinary Parasitology* **162**, 160–162.

Cowie, R. H. (2013*a*). Biology, systematics, life cycle, and distribution of *Angiostrongylus cantonensis*, the cause of rat lungworm disease. *Hawai'i Journal of Medicine and Public Health* **72**(Suppl. 2), 6–9.

Cowie, R. H. (2013*b*). Pathways for transmission of angiostrongyliasis and the risk of disease associated with them. *Hawai'i Journal of Medicine and Public Health* **72**(Suppl. 2), 70–74.

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

De Meuron, K. (2005). Angiostrongylose en Martinique: à propos de quatre cas pédiatriques. Ph.D. thesis. Faculte de Medecine, Université de Bourgogne, France. 134 pp.

Evans-Gilbert, T., Lindo, J. F., Henry, S., Brown, P. and Christie, C. D. (2014). Severe eosinophilic meningitis owing to *Angiostrongylus cantonensis* in young Jamaican children: case report and literature review. *Paediatrics and International Child Health* **34**, 148–152.

Giannelli, A., Colella, V., Abramo, F., do Nascimento-Ramos, R. A., Falsone, L., Brianti, E., Varcasia, A., Dantas-Torres, F., Knaus, M., Fox, M. T. and Otranto, D. (2015). Release of lungworm larvae from snails in the environment: potential for alternative transmission pathways. *PLoS Neglected Tropical Diseases* **9**, e0003722. doi: 10.1371/journal.pntd.0003722.

Heyneman, D., and Lim, B. L. (1967). *Angiostrongylus cantonensis*: proof of direct transmission with its epidemiological implications. *Science* **158**, 1057–1058.

Jovani, R. and Tella, J. L. (2006). Parasite prevalence and sample size: misconceptions and solutions. *Trends in Parasitology* **22**, 214–218.

Justine, J.-L., Winsor, L., Barrière, P., Fanai, C., Gey, D., Han, A. W. K., La Quay-Velazquez, G., Lee, B. P. Y.-H., Lefevre, J.-M., Meyer, J.-Y., Philippart, D., Robinson, D. G., Thévenot, J. and Tsatsia, F. (2015). The invasive land planarian *Platydemus manokwari* (Platyhelminthes, Geoplanidae): records from six new localities, including the first in the USA. *PeerJ* **3**, e1037.

King, C. H. (2001). PRO/AH/EDR> Eosinophilic meningitis – USA ex Jamaica. ProMED-mail post. International Society for Infectious Diseases. http://www.promedmail.org/direct.php?id=20011007.2426

Lindo, J. F., Waugh, C., Hall, J., Cunningham-Myrie, C., Ashley, D., Eberhard, M. L., Sullivan, J. J., Bishop, H. S., Robinson, D. G., Holtz, T. and Robinson, R. D. (2002). Enzootic *Angiostrongylus cantonensis* in rats and snails after an outbreak of human eosinophilic meningitis in Jamaica. *Emerging Infectious Diseases* **8**, 324–326.

Lindo, J. F., Escoffery, C. T., Reid, B., Codrington, G., Cunningham-Myrie, C. and Eberhard, M. (2004). Fatal autochthonous eosinophilic meningitis in a Jamaican child caused by *Angiostrongylus cantonensis*. *American Journal of Tropical Medicine and Hygiene* **70**, 425–428.

Lindo, J. F., Waugh, C., Todd, C., Brown, P. and Robinson, R. D. (2011). The emergence of *Angiostrongylus cantonensis* as a cause of eosinophilic meningitis in Jamaica: progress and pitfalls. In *Angiostrongylus and Angiostrongyliasis: Advances in the Disease, Control, Diagnosis, and Molecular Genetics* (ed. Eamsobhana, P.), pp. 27–31. Wattanakij Panich Press, Bangkok. ISBN 7978-974-11-1436-8.

Louisiana Office of Public Health (2006). *Angiostrongylus cantonensis*. Infectious Disease Epidemiology Section – Annual Report. http://www.dhh.state.la.us/assets/oph/Center-PHCH/Center-CH/infectious-epi/Annuals/LaIDAnnual_Angiostrongylus.pdf

Lv, S., Zhang, Y., Steinmann, P., Yang, G.-J., Yang, K., Zhou, X.-N. and Utzinger, J. (2011). The emergence of angiostrongyliasis in the People's Republic of China: the interplay between invasive snails, climate change and transmission dynamics. *Freshwater Biology* **56**, 717–734.

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

Mahon, R. and Aiken, K. (1977). The establishment of the North American Bullfrog, *Rana catesbeiana* (Amphibia, Anura, Ranidae) in Jamaica. *Journal of Herpetology* **11**, 197–199.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M. and Schad, G. A. (1982). The use of ecological terms in parasitology (report of an *ad hoc* committee of the American Society of Parasitologists). *Journal of Parasitology* **68**, 131–133.

Mattis, A., Mowatt, L., Lue, A., Lindo, J. F. and Vaughn, H. (2009). Ocular angiostrongyliasis - first case report from Jamaica. *The West Indian Medical Journal* **58**, 383–385.

Murphy, G. S. and Johnson, S. (2013). Clinical aspects of eosinophilic meningitis and meningoencephalitis caused by *Angiostrongylus cantonensis*, the rat lungworm. *Hawai'i Journal of Medicine and Public Health* **72**(Suppl. 2), 35–40.

Pascual, J. E., Bouli, R. P. and Aguiar, H. (1981). Eosinophilic meningoencephalitis in Cuba, caused by *Angiostrongylus cantonensis*. *American Journal of Tropical Medicine and Hygiene* **30**, 960–962.

Poulin, R. (1993). The disparity between observed and uniform distributions: a new look at parasite aggregation. *International Journal for Parasitology* **23**, 937–944.

Qvarnstrom, Y. J., Bishop, H. and da Silva, A. (2013). Detection of rat lungworm in intermediate, definitive and paratenic hosts obtained from environmental sources. *Hawai'i Journal of Medicine and Public Health* **72**(Suppl. 2), 63–69.

Raccurt, C. P., Blaise, J. and Durette-Desset, M. C. (2003). Presence of *Angiostrongylus cantonensis* in Haiti. *Tropical Medicine and International Health* **8**, 423–426.

Reiczigel, J. (2003). Confidence intervals for the binomial parameter: some new considerations. *Statistics in Medicine* **22**, 611–621.

Robinson, B. A., Tolan, W. and Golding-Beecher, O. (1990). Childhood pica. Some aspects of the clinical profile in Manchester, Jamaica. *West Indian Medical Journal* **39**, 20–26.

Robinson, R. D., Waugh, C. A., Todd, C. D., Lorenzo-Morales, J. and Lindo, J. F. (2013). Rat lungworm: an emerging zoonosis in Jamaica. *Hawai'i Journal of Medicine and Public Health* **72**(Suppl. 2), 33 (Refereed Abstract).

Rosenberg, G., and Drumm, D. (2004). Interactive key to Jamaican land snails. http://data.acnatsci.org/jamaica/keyinfo.html.

Rosenberg, G. and Muratov, I. V. (2006). Status Report on the terrestrial mollusca of Jamaica. *Proceedings of the Academy of Natural Sciences of Philadelphia* **155**, 117–161.

Rózsa, L., Reiczigel, J. and Majoros, G. (2000). Quantifying parasites in samples of hosts. *Journal of Parasitology* **86**, 228–232.

Slom, T. J., Cortese, M. M., Gerber, S. I., Jones, R. C., Holtz, T. H., Lopez, A. S., Zambrano, C. H., Sufit, R. L., Sakolvaree, Y., Chaicumpa, W., Herwaldt, B. L. and Johnson, S. (2002). An outbreak of eosinophilic meningitis caused by *Angiostrongylus cantonensis* in travelers returning from the Caribbean. *New England Journal of Medicine* **346**, 668–675.

Spratt, D. M. (2015). Species of Angiostrongylus (Nematoda: Metastrongyloidea) in wildlife: a review. *International Journal for Parasitology: Parasites and Wildlife* **4**, 178–189.

Thiengo, S. C., Simões Rde, O., Fernandez, M. A. and Maldonado, A. (2013). *Angiostrongylus cantonensis* and rat lungworm disease in Brazil. *Hawaii Journal of Medicine and Public Health* **72** (Suppl. 2), 18–22.

Vargas, M., Gomez Perez, J. D. and Malek, E. A. (1992). First record of *Angiostrongylus cantonensis* (Chen, 1935) Nematoda: Metastrongylidae in the Dominican Republic. *Tropical Medicine and Parasitology* **43**, 253–255.

Wallace, G. D. and Rosen, L. (1966). Studies on eosinophilic meningitis. 2. Experimental infection of shrimp and crabs with *Angiostrongylus cantonensis*. *American Journal of Epidemiology* **84**, 120–131.

Waugh, C. A., Shafir, S., Wise, M., Robinson, R. D., Eberhard, M. and Lindo, J. F. (2005). Human *Angiostrongylus cantonensis*, Jamaica. *Emerging Infectious Diseases* **11**, 1977–1978.

Waugh, C. A., Lindo, J. F., Forona, P., Angeles-Santana, M., Lorenzo-Morales, J. and Robinson, R. D. (2006). Population distribution and zoonotic potential of gastrointestinal helminths of wild rats *Rattus rattus* and *R. norvegicus* from Jamaica. *Journal of Parasitology* **92**, 1014–1018.

Webster, J. P., Brunton, C. F. and MacDonald, D. W. (1994). Effect of *Toxoplasma gondii* upon neophobic behaviour in wild brown rats, *Rattus norvegicus*. *Parasitology* **109**, 37–43.

Wilson, B. S. (1991). Latitudinal variation in activity season mortality rates of the lizard *Uta stansburiana*. *Ecological Monographs* **61**, 393–414.

Wong, M. S., Bundy, D. A. and Golden, M. H. (1991). The rate of ingestion of *Ascaris lumbricoides* and *Trichuris trichiura* eggs in soil and its relationship to infection in two children's homes in Jamaica. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 89–91.

York, E. M., Butler, C. J. and Lord, W. D. (2014). Global decline in suitable habitat for *Angiostrongylus* (= *Parastrongylus*) *cantonensis*: the role of climate change. *PLoS ONE* **9**, e103831.

Zhang, R.-L., Chen, M.-X., Gao, S.-T., Geng, Y.-J., Huang, D.-N., Liu, J.-P., Wu, Y.-L., and Zhu, X.-Q. (2008). Enzootic angiostrongyliasis in Shenzhen, China. *Emerging Infectious Diseases* **14**, 1955–1956.