Informational Briefs from the 6th International Workshop on Rat Lungworm Parasites and Diseases

Based on papers published in: *Parasitology*, Vol 148 (2) 2021

A natural depiction of an integral part of the life cycle of *Angiostrongylus canonensis* with a rat (*Rattus rattus*) (definitive host) eating a snail (intermediate host) in the Hawaiian forest.

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Compilers and Scientific Interpreters: Sue Jarvi and Kay Howe

Why this publication?

This publication came about as a result of research presented at the 6th International Workshop on Angiostrongylus and Angiostrogyliasis (colloquially known as rat lungworm disease) held in Hilo, HI, in January of 2020. We have published the scientific details of many of these studies in a Special Issue of Parasitology (Feb 2021) Vol. 148 (Coordinating Editor JT Ellis, Guest Editor SI Jarvi). Prior International Workshops have been held in Thailand (2010), Hawai'i (2011), China (2013), Australia (2016), then again in Thailand (2017), and Hawai'i in (2020). These Workshops bring together researchers from all over the world who are conducting the most cutting-edge research on a wide variety of topics related to this parasite and the disease it causes. These workshops are crucial for advancing research and knowledge not only about diagnosis and treatment, but on research relating to infection and transmission levels and the many influencing factors involved, as well as documenting the seemingly expanding range of infection in non-human species. Perhaps most importantly, research on ways of reducing ones risk of infection in the first place is critical, including the development of mechanisms and tools for enhanced public education. We feel that the results of these scientific studies should not be limited to those familiar with 'science-speak', but to be more inclusive and provide a less technical interpretation of these findings which is accurate, informative, and reader-friendly for the general population. Members of the general population are, in fact, all at risk from this potentially deadly disease, thus we are providing this publication free-of-charge to those interested. In addition, we are also providing a new online education program on the prevention of rat lungworm disease which is now available to the public, and includes CE credits for healthcare professionals. This course is taught by us (Dr. Sue Jarvi and Kay Howe, MS), and this 1.25 hour activity provides current information on the parasite's life cycle and how one can reduce the risk of infection. For more information and to register for the education program, go to: https://pharmacy.uhh.hawaii.edu/academics/continuing-education/rat-lungworm. We appreciate your interest in this fascinating but potentially deadly parasite, and please contact us with any questions or concerns (email: rlw411@hawaii.edu). Mahalo,

Sue Jarvi, PhD, and Kay Howe, MS

University of Hawai'i at Hilo and the Hawai'i Island Rat Lungworm Working Group

Neuroangiostrongyliasis (Rat Lungworm Disease) caused by *Angiostrongylus cantonensis*: A Brief Overview

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Overview and life cycle

Neuroangiostrongyliasis (NAS), infection with the rat lungworm, *Angiostrongylus cantonensis*, is the leading cause of human eosinophilic meningoencephalitis globally. The parasite's geographic range continues to expand from its presumed origin in Southeast Asia. It can infect and develop in other warm-blooded animals, including non-human primates, dogs, fruit bats, horses, marsupials, and some species of birds. However, it can reproduce only in its definitive hosts, several species of rats. Adult worms live in the right ventricle and pulmonary arteries, where females release eggs straight into the bloodstream. These lodge in alveolar capillaries, where they embryonate. Once the first-stage larvae (L1s) develop, they hatch, crawl out into the air spaces, and are carried in mucus up the bronchial tree, to be swallowed, passed through the gut and out in the rat's feces. The L1s need to develop to the third stage (L3) before they can infect another rat (Fig.1). This can happen only in the tissues of intermediate gastropod hosts, a wide range of slugs and snails. These gastropods usually acquire L1s by ingesting rat feces, though some (e.g. aquatic snails) might also be infected through their skin. The life cycle is completed when a rat eats the infected gastropod; L3s are released in the gut, then develop through the L4 and L5 stages in the central nervous system (CNS), before returning to the right ventricle of the heart, and pulmonary arteries, where they mature and reproduce.

Figure 1. *Angiostrongylus cantonensis* L3 isolated from a *Parmarion martensi* collected on East Hawai'i Island. (40X, Photo by J. Jacob)

Transmission

Most human infections are derived from gastropods; a less significant source is provided by various cold-blooded, paratenic hosts (such as freshwater shrimps, frogs, centipedes, or monitor lizards), which can harbor L3s after ingesting gastropods. Another likely transmission source is drinking water contaminated with L3s released by decomposing molluscs. The role of gastropod mucus deposited on vegetable greens has not yet been confirmed. In established endemic areas where domestic cultivation and consumption of snails are routinely practiced, such as parts of SE Asia, NAS can assume epidemic proportions. Elsewhere, it tends to occur sporadically, with a seasonal influence possibly reflecting gastropod behaviour. Except for cases of wilful slug/snail ingestion, the precise source of infections is rarely identified. Individual protection is essential for prevention, and depends on public health education. Care to avoid the ingestion of slugs and snails cannot be over-emphasised. Thoroughly cooking all food and boiling all drinking water will kill the L3s, assuring better protection.

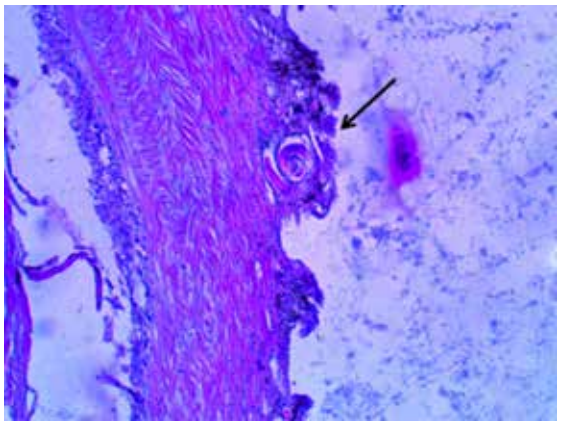

Figure 2. Histological section showing an *A. cantonensis* larva very close to the outside edge of the body wall of a *Parmarion martensi.*(10X, Photo by K. Howe)

Clinical manifestations

Human disease begins when ingested L3s invade the lining of the gastrointestinal tract. Symptoms such as pain, nausea, vomiting and diarrhea can occur within hours. Skin rashes have been reported. Most larvae reach the CNS via the circulation, although some might use alternative routes. In natural infections, an ingested gastropod might not break down fully until well down the gut, even as far as the colon, perhaps allowing L3s to invade pelvic lymphatics and/or nerves, as a gateway into the lower spinal cord. Symptoms of CNS invasion usually commence a week or more after exposure. The parasite does not multiply in humans, but does migrate extensively while growing hugely in size over a period of several weeks (about 15 mm long when leaving the CNS). Depending on the number of larvae and the locations through which they move, the manifestations of disease can fluctuate and vary remarkably and unpredictably, but they tend to worsen progressively, before gradually settling down.

Headache is common and probably caused by meningeal inflammation and elevated CSF pressure, the latter explaining the temporary relief often provided by lumbar puncture. Tissue damage results from a combination of direct mechanical and chemical disruption. Many larvae are killed by the immuno-inflammatory response, but their death leads to the formation of dense, fixed inflammatory foci ("abscesses"). The more advanced the larva, the bigger the lesion and the longer it will take to resolve. The remains of an immature adult worm (L5) might persist in the CNS for months or possibly years, leading to the formation of permanent scar tissue. However, variable numbers of L5s will survive long enough to leave the CNS, via blood vessels, and reach the lungs. Significant lung complications can occur when large numbers of adult worms develop in the human pulmonary arteries. Some human autopsies have shown that adult worms matured sufficiently to copulate, but we don't know if this could have resulted in L1s being expelled in human feces.

Diagnosis and Treatment

To minimise CNS damage, while the L3s are still small (ca. 0.3 mm long at invasion), treatment should be started as soon as the diagnosis is considered. If given early, albendazole can effectively kill these larvae, so it should be started immediately. Should a child or adult be known to have ingested a potentially infected snail or slug, then pre-emptive therapy is mandatory to prevent L3s from even reaching the CNS. In such an emergency, were albendazole not immediately available, mebendazole or pyrantel pamoate (over-the-counter pinworm medicine) should be given. Because they kill worms only within the gut lumen, these drugs will not affect L3s that have already invaded deeper tissues, so should be followed up by albendazole treatment as soon as practical. Corticosteroids should be administered with albendazole to reduce inflammation. However, beyond three weeks post-exposure, when most of the worms have either died, or grown large and are preparing to leave the CNS, worm medications become counter-productive. Killing any remaining live L5s will produce additional large and growing CNS lesions. The big difficulty with this is precisely timing the stage of infection. Few patients can identify their point of exposure, providing only vague clues to when and how the larvae might have been consumed. Larval numbers, then, can only be guessed from how severely they are affected.

Another major challenge is that a reliable diagnosis, by finding antibodies or parasite DNA in CSF or blood, can take several weeks. Work on ultra-sensitive tests is progressing. Even the onset of eosinophilia in blood or CSF can be delayed, although it must be stressed that normal CSF should be absolutely free of eosinophils, and must be stained accordingly for examination. There is no "normal cut-off number", so detection of just one in a blood-free sample indicates pathology. An identifiable larva in the CSF (or an eye) offers conclusive evidence, but is rarely encountered outside highly endemic areas. Measuring the size of any recovered larva is essential, for it allows a reasonable estimation of the stage of infection. Given the risk of extending potentially irreversible CNS damage while awaiting diagnostic confirmation, anthelminthic treatment should be given purely on clinical suspicion: if the presentation is consistent with early NAS or if in an area where NAS is known to be endemic, such as Hawai'i, it should be started immediately. It should be noted that early, severe NAS, especially in young children, but sometimes adults as well, can resemble the more common Guillain-Barré syndrome, which has no specific treatment, but a better prognosis. Respiratory symptoms are a common feature in late NAS, and very likely indicate lung involvement. These should not be disregarded but monitored clinically and radiologically.

Long-term sequelae

Despite even timely diagnosis and treatment, the nature of this disease will cause many patients to suffer prolonged, sometimes permanent, clinical sequelae. While some become convinced the parasite has survived, even multiplied, in their brain, the simple explanation is that their CNS lesions have been too extensive for complete resolution. They may be affected by irritating and debilitating symptoms indefinitely, with the only treatment available being non-specific, as offered by physiotherapy and pain clinics. It's perfectly understandable that some resort to "alternative" therapies that remain scientifically unconfirmed, purely out of desperation, although rarely with satisfaction. These long-term sequelae often lead to great frustration, with the superimposed burdens of financial hardship, lack of insurance, and strain on caregiving family and friends (or total lack of support). Given the paucity of detailed clinical follow-up in such chronic cases, let alone post-mortem studies, very little is known about the underlying pathology of late stage NAS.

This Special Summary contains informational briefs based on the cutting-edge research presented at the 6th International Workshop on *Angiostrongylus* and Angiostrongyliasis held in Hilo, Hawai'i, in January, 2020. The corresponding full-length papers were recently published in the international journal *Parasitology* **148 (2)**, 2021. As an overview, Niebuhr *et al*. (page 5), Paller *et al*.,(p7), and Walden *et al*., (p9) provide research updates on gastropod and rat infection levels under natural conditions in multiple geographic areas of the world, and Hamilton *et al*. (p11) and Wun *et al*. (p13) discuss infection levels under laboratory conditions. The latest research on infection in non-human species is described in birds by Feckova *et al*. (p15), in mice by Tsai *et al*. (p16), in non-human primates by Walden et al. (p9), and in dogs by Lee *et al*. (p17), and Wun *et al*. (p19). Modry *et al*. (p21) discusses alternative parasite transmission pathways via water. Methods for preventing infection or reducing risks of human exposure are addressed by Howe *et al*. (p23), and Steel *et al*. (p25). Long term effects of infection or chronic sequelae are described by Meyer (p27). Last, but certainly not least, updates on research in disease diagnosis and treatment are described by Ansdell *et al*. (p29), Eamsobhana *et al*. (p31), Jacob *et al*.(p33) and Atkinson *et al*. (p35). We hope these summarized briefs will provide reader-friendly information that might help to reduce the risk of infection in humans and other animals, and provide a better understanding to the general public of the current research being conducted on this parasite and disease.

Acknowledgements

We would like to thank all those who helped organize and make this meeting happen including Professor Gordon Ching, Rene Siracusa (posthumously), Vernon Ansdell MD, Karen Pellegrin PhD, Eileen O'hara, Patricia Macomber, UHH undergraduate and pre-med students (especially Victoria Rapoza and Nicole "Pua" Garza), and members of the Jarvi lab who helped in all aspects (Kay Howe MS, John Jacob PharmD, Lisa Kaluna MS, Kirsten Snook MS, Argon Steel PhD, and Yaeko Tagami). We would like to thank Tanya Ibarra for assistance with graphics, and Sunny Walker for assistance with the website. We thank Hawai'i Island legislators Senator Russell Ruderman, Representative Richard Creagan MD and Representative Chris Todd, as well as Lt. Governor Josh Green MD, and Senator Kai Kahele for their long-term support of our work through research and education to make the state of Hawai'i a safer place to visit and live. Mahalo!

Funding

Hawai'i State Legislature, *National Institutes of Health, National Institute of General Medical Sciences, IDeA Networks of Biomedical Research Excellence (P20GM103466), Gordon Ching and community members,* Hawai'i County Research and Development, Hawai'i County Council, Hilo Medical Center, Hilo, HI, Malama O Puna, Pahoa, HI, and Hawai'i Department of Health .

Original article: Jarvi S, Prociv P (2021). *Angiostrongylus cantonensis* and neuroangiostrongyliasis (rat lungworm disease):2020 Parasitology **148 (2)**, 129-132.

Dr. Chris Niebuhr is a vertebrate ecologist and research scientist for Manaaki Whenua - Landcare Research in Lincoln, New Zealand. He leads research efforts in conservation biology, invasive species managment and disease ecology in New Zealand and elsewhere in the Pacific. His postdoctoral research as a biologist for the USDA National Wildlife Research Center Hawai‘i Field Station in Hilo, Hawai‘i focused on invasive rats and mongooses in island ecosystems. Now in New Zealand, he continues to collaborate with researchers in Hawai‘i, Australia, and elsewhere on investigations into the epidemiology of rat lungworm disease.

Differences in rat lungworm infection levels in wild rats and snails in Hawai‘i

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Rat lungworm (*Angiostrongylus cantonensis*) is a tropical and subtropical parasitic nematode, with infections in humans causing rat lungworm disease (angiostrongyliasis). Hawai‘i has been identified as a global hotspot of infection, with recent reports of high infection rates in humans (accidental hosts), as well as wild rats (definitive hosts) and snails (intermediate hosts). The presence of both rats and snails are required to complete the life cycle of the parasite. This study investigated differences in the infection levels of two rat species and one snail species on Hawai‘i Island (Big Island). The two rats species studied here are *Rattus rattus* (known as the black, ship, or roof rat) and *Rattus exulans* (known as the Pacific or Polynesian rat), both of which have been introduced to Hawai‘i. The snail species studied here is *Parmarion martensi* (often referred to as a “semi-slug”, although slugs and semi-slugs are all technically types of ‘snails’), which has also been introduced to Hawai‘i. We sampled a total of 417 rats (191 *R. rattus* and 226 *R. exulans*) and 159 snails, from two sites near Hilo, Hawai‘i, to better understand the infection levels occurring in rats and snails living in the wild. Two different measurements of infection were considered, including ‘infection prevalence’ (or the proportion of a group that is infected, which ranges from 0-100%) and ‘infection intensity’ (or the average number of parasitic worms found per animal). For the rats, we counted the number of adult worms found in the heart and lungs. For the snails, we used molecular techniques in the laboratory to estimate the number of worm larvae in snail muscle tissue. Finally, we used statistical modelling to better investigate the infection levels observed here.

In our study, we found 86% of the snails and 64% of the rats were positive for the rat lungworm parasite. However, we found these infection levels varied, depending on which rats were sampled (including the rat species, the size of the rat, and even the sex of the rat). The Pacific rats (77.4%) were more infected than the black rats (47.6%), as well as having a higher worm burden. We also found that while the Pacific rats were more infected the larger they got (likely due to more snails eaten over time), the black rats showed the opposite. We hypothesise that the black rats may be acquiring a certain level of immunity to the worms over time, while the other rat species is not. If this is true, then it may be important when planning any rat control, as one species may be more important than the other in terms of the parasite’s life cycle. We also found that larger snails were more infected than smaller snails. Additionally, we also found that certain environmental factors influence the levels of infection. For example, we found that, while infections in black rats varied greatly throughout

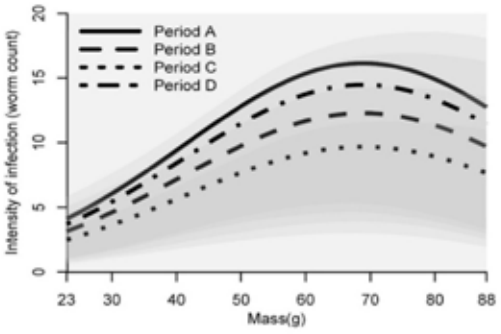

Figure 1. Reproduced with permission from Niebuhr et al. (2021). Variation in *Angiostrongylus cantonensis* (rat lungworm) infection intensity for *Rattus exulans* (Pacific rat) among four sampling periods. Periods A–D represent host sampling periods (A: May 2018; B: Aug.–Sep. 2018; C: Nov.–Dec. 2018; D: Feb. 2019)

the year, infection in Pacific rats (both prevalence and intensity) were higher in February and May, than around August and November (see Figure 1 below). However, since our data only comes from a single year, it is difficult to accurately determine an annual cycle of infection. The May sampling period also showed the highest infection levels in snails tested, with a trend of decreasing levels for the subsequent sampling months, but only at one of the two sites (see Figure 2 below). The other site showed more variability, which shows location, as well as timing, is important to infection levels. Information on sources of variability of infection in wild host populations will be a crucial component in predicting the effectiveness of future disease surveillance or targeted management strategies.

Original article: Niebuhr, CN, Siers, SR, Leinbach, IL, Kaluna, LM and Jarvi, SI. 2021. Variation in *Angiostrongylus cantonensis* infection in definitive and intermediate hosts in Hawai‘i, a global hotspot of rat lungworm disease. *Parasitology* **148 (2),**133–142. doi: 10.1017/S003118202000164X

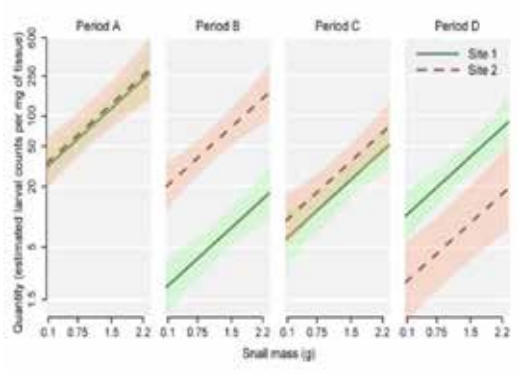

Figure 2. Reproduced with permission from Niebuhr et al. (2021). Infection intensity of *Angiostrongylus cantonensis* (rat lungworm) infection in *Parmarion martensi* snails (or semi-slugs) sampled over four sampling periods at two sites in the vicinity of Hilo, Hawai‘i. Periods A–D represent host sampling periods (A: May 2018; B: Aug.–Sep. 2018; C: Nov.–Dec. 2018; D: Feb. 2019.)

Dr. Vachel Gay V. Paller is a Professor of the Institute of Biological Sciences, University of the Philippines Los Baños, Philippines. Parasitology was her field of specialization for her doctoral degree from Kobe University at the Graduate School of Health Sciences in Japan. She is active in teaching, research, and extension projects but her passion is to do science to help improve the lives of the Filipino people. Dr. Paller believes parasitology is a field needing more attention in developing tropical countries where parasites are widespread.

Angiostrongylus cantonensis in non-native rats in Philippine Wildlife Ecosystem: a case of potential agent of zoonotic pathogen spillover

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This study provides important information on the extent of *Angiostrongylus cantonensis* infection of non-native rats in Philippine Mount Makiling Forest Reserve (MMFR) and adjacent areas (Fig 1). MMFR was recorded to have high mammal species diversity, including native and non-native rats, which are the most diverse group in the area (Laguna De Bay Environgmental Action Planning, 2005). Three species of non-native rats, including (Fig 2A) the brown or Norway rat *Rattus norvegicus* (Fig 2B) *Rattus tanezumi* (field rats) and (Fig 2C), *Rattus exulans* (Pacific rat), were found in MMFR and its adjacent agricultural and residential areas. These rats were found to be infected with *Angiostrongylus cantonensis* (Estaño, et al., 2020). Moreover, this study also revealed that rats from agricultural and agro-forest areas showed significantly higher infection rates than those from residential areas. The recovery of *A. cantonensis* from the non-native rat species in MMFR and its adjacent areas has implications for public health, posing a potential spillover of pathogens from wildlife and agriculture to nearby areas converted for human habitations.

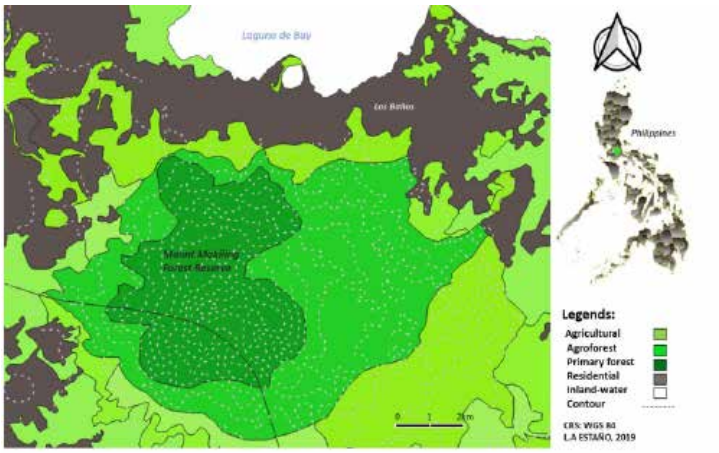

Figure 1. Map of the Philippine Mount Makiling Forest Reserve and surrounding areas involved in this study.

Figure 2. Non-native rats found in MMFR and its adjacent agricultural and residential areas include (A) the brown or Norway rat *Rattus norvegicus,* (B) *Rattus tanezumi* (field rats), and (C) *Rattus exulans* (Pacific rat).

There are several factors that could influence the spread of *A. cantonensis*, such as animal movement, human behavior, environmental changes, and interactions of these factors. In the Philippines, native and non-native rats are abundant in various ecosystems such as wild forests, agriculture, and residential areas. MMFR is a semi- pristine area, however, due to increased human activities, its foothill and adjacent areas are being utilized for agroforestry and partly converted into residential and ecotourism areas. These anthropogenic usages, such as human settlements,

tourism and agricultural activities, have brought partial environmental degradation of the area. This has also led to the change in population dynamics of wildlife including non-native rat species. Moreover, the movement and possible encroachment of native and non-native rats to human habitations could lead to possible spillover of zoonotic agents, posing threats to public health.

Understanding the transmission dynamics of *A. cantonensis* involving rats and other wild animals, its intermediate and other reservoir hosts, vis-à-vis human behavior, and climate and environmental changes, are significant in the control and prevention of zoonotic health threats. With urbanization and the everchanging landscape of MMFR watersheds and buffer zones, there is a need to conduct monitoring and surveillance of wildlife pathogens to prevent zoonotic disease spillover.

Original article: L.A Estaño, AMD Bordado, VGV Paller (2021). *Angiostrongylus cantonensis* Infection of Non-native Rats in Mount Makiling Forest Reserve, the Philippines. *Parasitology* **148 (2),** 143-148.

Dr. Heather D. S. Walden is an Assistant Professor of Parasitology at the University of Florida, College of Veterinary Medicine at Gainsville, Florida. Her focus is on Zoonotic parasitic disease, diagnosis, and classical parasite biology. The laboratory works with parasites of exotic and domestic hosts of all taxonomic groups ranging from fish, amphibians, reptiles and birds, to mammals including marine and non-human primates. She is currently working with zoonotic metastrongyloid nematodes, specifically *Angiostrongylus cantonensis* and its geographical distibution and host species throughout Florida.

Angiostrongylus cantonensis (rat lungworm) in Florida, USA

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Angiostrongylus cantonensis has been found in Florida, USA, from the panhandle in the north to Miami and surrounding areas in the southern parts of the state. A limited study completed in 2015 found evidence of *A. cantonensis* in definitive rat hosts, intermediate gastropod hosts, and an armadillo. Additional studies have identified this parasite in a variety of intermediate hosts, both native and non-native gastropod species, with new host species recorded. Case reports in the state currently involve non-human primates and include a gibbon and orangutan in Miami. Here, we report current status of *A. cantonensis* in the state, as well as the infection in a capuchin monkey and presumptive infection in a red ruffed lemur in Gainesville, FL. Each non-human primate presented similarly. The white handed gibbon, housed at the Miami Metro Zoo in Miami, FL, was reported to have extreme limb weakness with nematodes recovered from the brain and spinal cord. The orangutan, also from Miami, presented with pelvic limb weakness and fever, had minimal function of her limbs and muscle atrophy. Lumbar spinal fluid showed an increase in eosinophils (86%). The orangutan had a history of eating snails. The white throated capuchin monkey and red ruffed lemur in Gainesville, FL presented with similar signs including acute onset of lethargy and muscle weakness. Moderate increased eosinophil counts were noted (55% and 45%, respectively), and the capuchin was definitively diagnosed by a polymerase chain reaction (PCR) test of the cerebral spinal fluid (CSF) sample. Both the capuchin monkey and lemur were treated with fenbendazole and recovered.

Location (county)	Species name	Common name	Confirmation of infection
NON-HUMAN PRIMATES			
Alachua	*Cebus capucinus*	White-throated capuchin monkey	PCR
	Varecia rubra	Red ruffed lemur	History, location, presumptive diagnosis
Miami-Dade	*Hylobates lar*	White-handed gibbon	Morphological characteristics (gross parasite), PCR
	Pongo pygmaeus	Orangutan	Morphological characteristics (histological), PCR
RODENT AND OTHER HOSTS			
Alachua	*Rattus rattus*	Black rat	Morphological characteristics (gross parasite), fecal exam, PCR

Hillsborough	*Rattus rattus*	Black rat	Morphological characteristics (gross parasite), fecal exam, PCR
	Dasypus novemcinctus	Nine-banded armadillo	PCR
Miami-Dade	*Rattus rattus*	Black rat	Fecal exam, PCR
Orange	*Rattus rattus*	Black rat	Morphological characteristics (gross parasite), fecal exam, PCR
St. Johns	*Rattus rattus*	Black rat	Fecal exam, PCR
MOLLUSK HOSTS – ALL identified by presence of infective larvae and/or PCR			
Alachua	*Bradybaena similaris*		
Hillsborough	*Paropeas achatinaceum, Succinia floridana, Zonitoides arboreus*		
Leon	*Ventridens demissus*		
Miami-Dade	*Lissachatina fulica, Zachrysia provisoria, Bradybaena similaris, Alcadia striata*		
Orange	*Zachrysia provisoria*		

Original article: Walden, H., Slapcinsky, J., Rosenberg, J., & Wellehan, J. (2021). *Angiostrongylus cantonensis* (rat lungworm) in Florida, USA: Current status. *Parasitology*, **148 (2),** 149-152. doi:10.1017/S0031182020001286

Lindsey Hamilton's early professional experiences include data management, botanical and wildlife monitoring, and native plant production. She has explored the socio-political and economic sides of environmental work, and advocated for social justice and conservation while coordinating an endangered butterfly rearing program within a state prision. In Hawai'i, she has worked on projects to help farmers with challenging pest problems including the coffee borer beetle. She is currently partnering with the Dr. Susan Jarvi lab to develop phytosanitary irradiation treatments for the semi slug *Parmarion martensi* and *A. cantonensis.*

Laboratory rearing and demographics of the semi-slug *Parmarion martensi*, an intermediate host for *Angiostrongylus cantonensis*

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The semi-slug, *Parmarion martensi*, is an intermediate host of the zoonotic nematode, *Angiostrongylus cantonensis*, that causes rat lungworm disease in humans. Recent studies in Hawai'i have used field-collected *P. martensi* as a source of *A. cantonensis* for experimentation; however, wild-caught individuals may also be carrying other nematode species. Evaluation of management tactics relies on performance comparisons between treated and untreated control groups, which can only be accomplished with a reliable rearing system. The USDA Agricultural Research Service partnered with the University of Hawai'i at Hilo, College of Pharmacy, to develop rearing methods for the semi-slugs to facilitate studies on nematode transmission and control.

Figure 1. *Parmarion martensi* egg cluster (left) at 1 day before emergence, showing developing neonates, and a mature adult (right).

Parmarion martensi exhibited high survivorship when reared on a diet of dog food and fresh fruits and vegetables in temperature-controlled cabinets at 70.5 °F, 98% relative humidity, and 12:12 Light:Dark cycle. Rearing containers consisted of ventilated plastic bins with moist paper towels for substrate and plastic pots for hiding/resting and egg-laying. *Parmarion martensi* reached reproduction age at 165.3 ± 12.3 days and produced about 34.5 ± 7.8 eggs per adult throughout their reproduction cycle. Of the eggs that were produced, 52.7 ± 3.2 % of them hatched. Newly hatched neonate *P. martensi* had a survival rate of 86.2 ± 2.9 % at 30 days and 99% thereafter for up to a year. Laboratory-reared and wild-caught *P. martensi* were similar except for the weight of reproductive adults, which was significantly higher in laboratory-reared adults (4.0 ± 0.2 g) than in field-collected adults (1.5 ± 0.1 g).

A healthy and parasite-free laboratory colony eliminates uncertainty in the infection status in test subjects and helps reduce the risk of worker exposure. Uninfected *P. martensi* can also be infected in a controlled manner with *A. cantonensis* or other pathogenic nematodes at known infection rates. This will help in the evaluation of control strategies such as UV, ozonation, and vegetable washes. A parasite-free colony will also facilitate research concerning the molecular and biochemical pathways of *P. martensi* immune reactions with *A. cantonensis*, as host-pathogen interactions may be crucial to the development of drugs, therapies, and control strategies for zoonotic parasitic nematodes. The semi-slug *P. martensi* is also considered a quarantine pest as it does not occur in the continental United States (U.S. mainland). Irradiation studies are needed to identify a treatment dose to stop reproduction in *P. martensi* and prevent any invasive establishment.

Original Article: Hamilton, L., Tagami, Y., Kaluna, L., Jacob, J., Jarvi, S., & Follett, P. (2021). Demographics of the semi-slug *Parmarion martensi*, an intermediate host for *Angiostrongylus cantonensis* in Hawai‘i, during laboratory rearing. *Parasitology* **148 (2),**153-158. doi:10.1017/S0031182020001353

Dr. Richard Malik currently works as a consultant for the Centre for Veterinary Education at the University of Sydney. He is known internationally for his expertise in infectious diseases. He was the first person to diagnose rat lungworm disease in dogs in Sydney in the early 1990s and has maintained an interest in neuroangiostrongyliasis since then. He has a reputation as one of the worlds‘‘most respected and well-known feline veterinarians”.

Descriptions of the gross, microscopic, radiologic, echocardiographic and haematological findings in rats experimentally infected with *Angiostrongylus cantonensis*

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Although the lung and heart pathology in rats infected with *Angiostrongylus cantonensis* has been well described, corresponding changes detected using modern diagnostic imaging modalities have not been reported. This work describes the changes in the heart and lungs of mature laboratory rats chronically infected with moderate burdens

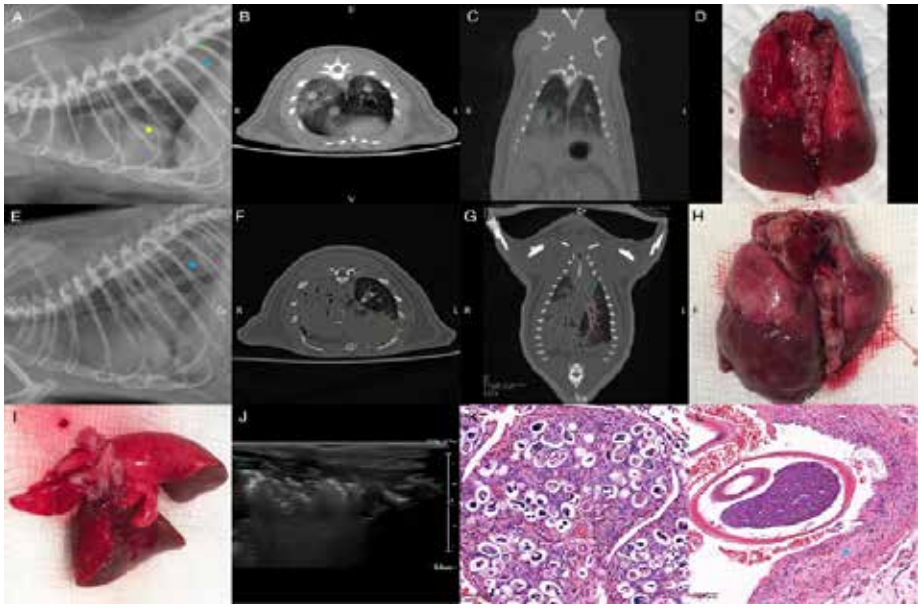

Fig. 1. (A) Radiograph. Evidence of lung fluid accumulation is present (blue star). The margins of the heart are poorly defined. (B) CT angiography image. The pulmonary arteries are markedly enlarged. A soft tissue opacity is present in the right lung lobe (blue arrows) and to a lesser degree in the left lung lobe (yellow arrows). Right pulmonary artery (a) together with bronchus (b) and pulmonary vein (v). (C) CT image. Pulmonary lobar arteries (artery branches in the lung lobes) are enlarged and do not taper normally (blue arrows). (D) Lungs at necropsy (autopsy). Note numerous dark areas, worse on the right. (E) Radiograph (X-ray). Evidence of fluid accumulation is present in the rear region (blue star). The margins of the heart are poorly defined. (F & G) CT images. The right lung lobe (blue arrows) appears fluid-filled with small remaining gas pockets associated with the bronchi. Enlarged left pulmonary artery (a), together with bronchus (b) and pulmonary vein (v). (H) Dorsal aspect of lungs after removal from the chest. Note the extensive areas of dark discolouration of the right rear lung lobe and rear aspect of the left lung lobe, more marked on the right. (I) Dissected lungs. Numerous mature lungworms can be seen in the left pulmonary arteries. (J) Ultrasound appearance of rear lung lobe regions. Note the complex heterogeneous echogenicity (indicating malignancy) and comet-tail artifact due to residual gas within the markedly abnormal lung tissue. (K & L) Microscopic images of infected lungs. (K) An inflammatory nodule consisting of parasitic eggs and first-stage larvae surrounded by inflammatory and fibrous cells. (L) Transverse (cross) sections of adult worms can be seen within a pulmonary artery. Note the thickened vascular walls (blue star).

of *A. cantonensis* using radiology, computed tomography (CT), CT angiography and echocardiography (ultrasound of the heart), correlated with post-mortem and microscopic examinations. Blood analysis and coagulation studies were also performed. Chest radiography (X-ray), CT and CT angiography (examination of blood vessels and organs) showed changes suggestive of fluid accumulation in the lung, mainly affecting the rear lung lobes, and associated dilatation of the arteries supplying those lung regions with blood. Presumptive worm profiles could be detected using echocardiography (ultrasound), with worms seen in the region in the heart pumping blood to the lungs. Numerous dark areas and multiple pale dot-like lesions affecting the rear lung lobes were observed at post-mortem. Microscopically, these were composed of numerous large, merging inflammatory and excessively fibrous nodules. Adult worms were found predominantly in the mid- to distal pulmonary arteries, carrying blood from the heart to the lungs. Blood analysis in most rats showed changes consistent with systemic inflammation. These findings provide a comparative model for *A. cantonensis* in its accidental hosts, such as humans and dogs. In addition, the pathological and imaging changes are comparable to those seen in dogs infected with *Angiostrongylus vasorum*, which is a related worm found in dogs and is of interest to veterinarians especially in the UK. We conclude that rats infected with *A. cantonensis* could be a model for dogs with *A. vasorum* infection.

Original article: Wun, M., Davies, S., Spielman, D., Lee, R., Hayward, D., & Malik, R. (2021). Gross, microscopic, radiologic, echocardiographic and haematological findings in rats experimentally infected with *Angiostrongylus cantonensis*. *Parasitology* **148 (2),** 159-166. doi:10.1017/S0031182020001420

A faculty of Veterinary Medicine at the University of Veterinary and Pharmaceutical Sciences in Brno, Czech Republic, Dr. Feckova has worked on the biology of metastrongylid nematode infections. Her career focus is on zoonotic parasites, species conservation, and education. She has worked in Spain, Romania, the Canary Islands, and the Philippines, and as a volunteeer veterinarian at the Cheetah Rescue Center Somaliland.

Pathology of *Angiostrongylus cantonensis* infection in two galliform bird species

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Angiostrongylus cantonensis causes severe neurological disorders in a wide range of warm-blooded animals, including several bird species. In tawny frogmouths and some species of cockatoos, fatal cases of neural angiostrongyliasis have been described in the scientific literature. Despite the apparent danger *A. cantonensis* infection may pose to them, there is a lack of experimental studies on birds. Given the occurrence of potential intermediate and paratenic hosts (snails, slugs, flatworms) in the diet of galliform birds, we aimed to investigate the susceptibility of domestic chicken and Japanese quail to *A. cantonensis* infection. After the experimental infection, we investigated the presence of larvae in the brain and spinal cord, described the clinical course of the early infection stage and assessed the relationship between larval dose and pathology.

The experimental strain of *Angiostrongylus cantonensis* used in this study originates from Fatu Hiva, French Polynesia and has been kept in laboratory conditions by circulating among laboratory rats and experimental snails as intermediate hosts. The infective L3 larvae were obtained from infected land snails and orally given to three groups of experimental birds per each species. Low dose groups of birds were infected by 100 infective L3 larvae, high dose groups by 1500 L3 larvae and the birds in the third group were fed three infected snails, mimicking a natural infection. The health status, food intake and behaviour of animals were observed during the first week after infection, blood tests and necropsy findings were used to assess the pathology of the infection. The values of experimental birds were compared to control birds, which received the same housing, food and treatment, but were not infected at the start of the experiment.

During the study, some of the infected birds showed an increase of the number of eosinophils in blood, while in others mild neurological signs, such as muscle twitching and unsteady gait, were observed. The infective dose did not seem to have an effect on those changes. No larvae were observed in microscopic examination of serial stained tissue sections of the brain and spinal cord of infected birds one week after infection. Also, no major gross lesions were observed during necropsy and microscopic examination of histological organ sections did not reveal lesions directly attributable to *A. cantonensis* infection.

The results of this study suggest that galliform birds are not highly susceptible to *A. cantonensis* infection. This opens a question of the importance of Galliformes in endemic areas as natural pest control, lowering the number of hosts carrying the infective larvae. Their role deserves more attention as a part of prevention of human infections.

Original article: Fecková, B., Djoehana, P., Putnová, B., Valašťanová, M., Petríková, M., Knotek, Z., & Modrý, D. (2021). Pathology of *Angiostrongylus cantonensis* infection in two model avian hosts. *Parasitology* **148 (2),**174-177. doi:10.1017/S0031182020001869

Dr. Tsai currently serves as the attending physician of Infectious Diseases Department at Kaohsiung Veterans General Hospital in Kaohsiung, Taiwan, and is an associate professor at the College of Medicine, National Yang-Ming University in Taipei. He studied the mechanism of blood brain barrier damage and worked as a research fellow at Saban Research Institute, Children's Hospital of Los Angeles and University of Southern California, USA from 2005-2006.

Mechanisms of the beneficial effects of dexamethasone in mice with eosinophilic meningitis caused by *Angiostrongylus cantonensis* infection

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Dexamethasone is a steroid that has been shown to have beneficial effects in patients and mice with eosinophilic meningitis caused by infection with the parasitic nematode *Angiostrongylus cantonensis*. In a mouse model, several weeks after infection with *A. cantonensis*, damage to the blood brain barrier (BBB) can result in cerebrospinal fluid (CSF) leakage and promote eosinophil infiltration into the brain, thus enhancing inflammation. This study evaluates the effects of dexamethasone on BBB integrity by studying the levels of proteins that are involved in the regulation and development of the central nervous system (e.g. MMP-9) and cellular signaling molecules involved in gene expression (e.g. NF-κB, JNK and ERK). This study shows that dexamethasone helps stabilize the BBB, therefore reducing the potential for eosinophil infiltration into the brain and leakage of CSF. There exists clear evidence that oxidative stress damages cells, proteins, and DNA (e.g. in the aging process). A well-established biomarker for oxidative stress in cells is 8-OHdG. This study shows that dexamethasone significantly downregulated the amounts of 8-OHdG in the CSF, thus reducing the potential for cellular damage. In summary, the administration of dexamethasone to patients infected with *A. cantonensis* reduces damage to the BBB, thereby reducing inflammation due to eosinophils and leakage of CSF, and also decreases cellular damage due to oxidative stress.

Original article: Tsai, H., & Chen, Y. (2021). Dexamethasone downregulates the expressions of MMP-9 and oxidative stress in mice with eosinophilic meningitis caused by *Angiostrongylus cantonensis* infection. *Parasitology* **148 (2),** 187-197. doi:10.1017/S0031182020001870

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Neuroangiostrongyliasis (rat lungworm disease) in Australian dogs: New cases (2010–2020) and results for a new, more-sensitive qPCR assay

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The principal aim of this study was to optimize the diagnosis of canine neuroangiostrongyliasis (rat lungworm disease). In total, 92 cases were seen between 2010 and 2020. Dogs were aged from 7 weeks to 14 years (median 5 months), with 73/90 (81%) less than 6 months and 1.7 times as many males as females. The disease became more common over the study period. Most cases (86%) were seen between March and July. Cerebrospinal fluid (CSF) was obtained from around the brain in 77 dogs, around the lower spinal cord in 5, and both sites in 3. Nucleated cell (lymphocytes, monocyte/macrophages, neutrophils, eosinophils) counts for 84 specimens ranged from 1 to 146150 cells per microliter (μL^{-1}) (median 4500). Percentage eosinophils (inflammatory cells sometimes used as marker for parasitic infections) varied from 0 to 98% (median 83%). When both brain and lower spine CSF were collected, inflammation was more severe caudally (near the tail).

Seventy-three CSF specimens were subjected to enzyme-linked immunosorbent assay (ELISA) testing for antibodies against *A. cantonensis*; 61 (84%) tested positive. Sixty-one CSF specimens were subjected to real-time quantitative polymerase chain reaction (qPCR) testing for *A. cantonensis* DNA using a new protocol targeting a bioinformatically-informed repetitive genetic target. The test is more senstive than the protocol currently used in PCR for detecting parasite DNA. Of 61 samples 53 (87%) tested positive. For 57 dogs, it was possible to compare CSF ELISA serology and qPCR. ELISA and qPCR were both positive in 40 dogs, in 5 dogs the ELISA was positive while the qPCR was negative, in 9 dogs the qPCR was positive but the ELISA was negative, while in 3 dogs both the ELISA and qPCR were negative. This

data suggests qPCR and ELISA testing in combination should confirm 95% of canine cases using a single CSF sample. A negative PCR and/or ELISA result does not exclude neuroangiostrongyliasis, however, as the sample might have been obtained before sufficient DNA or antibodies was released into the CSF. Neuroangiostrongyliasis is an emerging infectious disease of dogs in Sydney, Australia.

Original article: Lee, R., Pai, T., Churcher, R., Davies, S., Braddock, J., Linton, M., Yu, J., Bell, E., Wimpole, J., Dengate, A., Collins, D., Brown, N., Reppas, G., Jaensch, S., Wun, M.K., Martin, P., Sears, W., Šlapeta, J., & Malik, R. (2021). Further studies of neuroangiostrongyliasis (rat lungworm disease) in Australian dogs: 92 new cases (2010–2020) and results for a novel, highly sensitive qPCR assay. *Parasitology* **148 (2),**178-186. doi:10.1017/S0031182020001572

Matthew Wun will finish a Doctorate of Veterinary Medicine from the Sydney School of Veterinary Science at the University of Sydney, Australia. He has an interest in basic and clinical research and is currently involved in a number of rat lungworm studies with Dr. Richard Malik. During his degree he has won research scholarships at the Sydney Pharmacy School and Cornell Leadership Program for Veterinary Students.

Using magnetic resonance imaging (MRI) in dogs affected with neuroangiostrongyliasis (rat lungworm disease)

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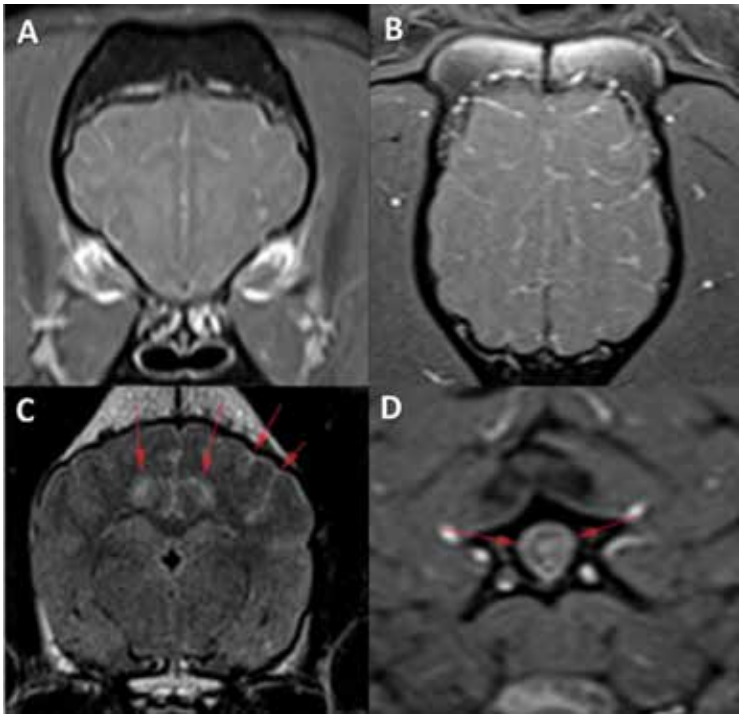

Fig. 1. MR images from a 14-month-old French Bulldog with a definitive diagnosis of neuroangiostrongyliasis and clinical signs suggestive of brain disease. The transverse (A) and dorsal (B) plane of the cerebral hemispheres show the prominence and increased enhancement of the brain lining in both cerebral hemispheres. A transverse image of the parietal lobes is shown in (C). Note the hyperintensity associated with the brain lining (red arrows), particularly in the left parietal lobe. Transverse image of the cervical spine is presented in (D); note the ring of contrast enhancement surrounding the spinal cord (red arrows).

The magnetic resonance imaging (MRI) appearance of the brain and spinal cord in humans with neuroangiostrongyliasis (rat lungworm disease) due to *Angiostrongylus cantonensis* has been well reported. Equivalent studies in animals are lacking. This case series describes clinical and MRI findings in 11 dogs with presumptively or definitively diagnosed neuroangiostrongyliasis. MRI of the brain and/or spinal cord was performed using various scan protocols. In four out of six cases where the brain was imaged, changes consistent with diffuse inflammation of the brain tissue and lining were observed. The spinal cord was imaged in nine dogs, with evidence of inflammation of the spinal cord lining and spinal cord tissue detected in regions consistent with physical exam findings. Characteristic changes of larvae migration through the nervous tissue, such as worm tracks seen in some human patients with neuroangiostrongyliasis, were not detected. Neuroangiostrongyliasis should be considered in the differential diagnosis of dogs with MRI evidence of focal or diffuse inflammation of the brain, spinal cord and/or brain or spinal cord lining, especially in areas where *A.*

cantonensis is endemic. If not ruled out in advance by imaging findings suggestive of brain herniation, cerebrospinal fluid (CSF) collection for cellular diagnostic purposes, fluid analysis, and qPCR and ELISA testing should be considered mandatory in such cases after the MRI studies.

Original article: Wun, M., Malik, R., Yu, J., Chow, K., Lau, M., Podadera, J., Webster, N., Lee. R., Šlapeta, J., & Davies, S. (2021). Magnetic resonance imaging in dogs with neuroangiostrongyliasis (rat lungworm disease). *Parasitology* **148 (2),**197-205. doi:10.1017/S0031182020001742

Dr. David Modry is Department Head of Parasitology and Pathology at the University of Veterinary Pharmaceutical Sciences Brno, Czech Republic, and Senior Researcher, Biology Center of Czech Academy of Sciences. He is interested in the complexity of parasite-host interactions in the context of ecosystems, particularly the transmission of infections at the human/wildlife/domestic animal interface. Dr. Modry and his team investigate peculiarities of the life cycle of *A. cantonensis* in isolated island ecosystems as well as the risk of introduction of the parasite into European terrritory.

Alternative transmission pathways of *Angiostrongylus cantonensis*

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Usually, the infection of the vertebrate hosts (including humans) by *Angiostrongylus cantonensis* occurs by ingestion of L3 through consumption of molluscs (obligate intermediate hosts) as well as of amphibians and reptiles as paratenic hosts, or crustaceans or arthopods as transport hosts. However, the importance of free-living L3 escaping the intermediate host either while alive or after its death was demonstrated for related metastrongylid nematodes of carnivores and repeatedly discussed also in case of transmission of *A. cantonensis* to humans. Indeed, when terrestrial gastropods drown in an aquatic environment, L3 may spontaneously emerge into the water. In order to elucidate the infection pathways of third stage larvae (L3) of *A. cantonensis* we performed experiments to assess: (i) the shedding of L3 from two species of experimental slugs drowned in water and the ratio of emerged larvae, (ii) the transmission of viable L3 from drowned terrestrial gastropods to aquatic snails, and, (iii) the transmission of viable L3 between terrestrial snails. Molluscs were experimentally infected by first stage larvae (L1) of *A. cantonensis*. Significantly more L3 larvae were released from *Veronicella cubensis* than from *Veronicella sloanei.* Numerous L3 were observed in the muscular foot, and also in the connective tissue between internal organs. Experimental exposure of aquatic snails *Pomacea maculata* to L3 of *A. cantonensis* liberated from other gastropod species led to their infection and the infectivity of larvae after intermediesis was demonstrated by infection of laboratory rats. The transmission of L3 was observed in three out of four experiment replications and L3 were retrieved from 6 out of 24 *Subulina octona* snails.

- We experimentally proved massive escape of third stage larvae of *A. cantonensis* from bodies of dead snails or slugs
- Spontaneously released L3 were able to infect other molluscs both in aquatic and terrestrial systems
- The L3 after intermediesis are able to cause patent infection in rats as definitive hosts

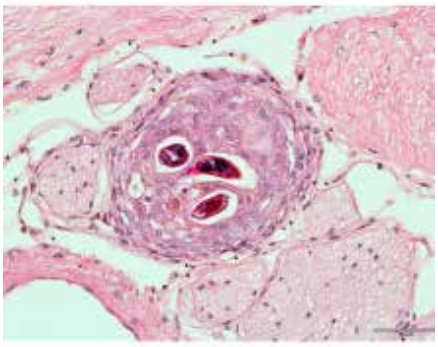

Fig. 1: Histopathological section showing L3 larvae in in tissues of experimental slugs infected in experiment.

The infected molluscs living in close association with humans represent a key-component in the epidemiology of human infections by *A.*

cantonensis. Escape of L3 larvae from bodies of dead snails or slugs and their ability to infect further gastropod hosts (intermediesis) represents a public health risk. Thus, control of molluscs living in peri-domestic environment is essential part of prevention of human infections.

Original article: Modrý, D., Fecková, B., Putnová, B., Manalo, S., & Otranto, D. (2021). Alternative pathways in *Angiostrongylus cantonensis* (Metastrongyloidea: Angiostrongylidae) transmission. *Parasitology* **148 (2),**167-173. doi:10.1017/S0031182020001857

Kathleen Howe has worked with the Jarvi lab at the University of Hawai‘i, Hilo, Daniel K. Inouye College of Pharmacy since 2012. While involved in many research studies, her primary focus has been to develop rat lungworm STEAM (science, technology, engineering, art, math) curriculum for K-12 schools. Teachers and their students assist researchers as citizen scientists in data collection relating to gastropod population dynamics and efffectivity of control measures, and become community educators for rat lungworm disease prevention.

A Hawai‘i community education program for the prevention of rat lungworm disease

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Public education is essential if Hawai‘i is to lower cases of neuoroangiostrongyliasis, commonly called rat lungworm disease (RLWD), that are occuring annually. The Jarvi Lab at the University of Hawai‘i, Hilo, Daniel K. Inouye College of Pharmacy has been involved with this effort since 2012. It recently partnered with the Hawai‘i Public Health Institute‘s, Hawai‘i Farm to School Hui to provide educational opportunties for K-12 schools statewide to learn more about the rat lungworm (RLW) and RLWD prevention.

A for-credit course on RLWD prevention was offered to Hawai‘i K-12 teachers during the 2018 - 2019 school year. Fourteen teachers, eleven from Hawai‘i Island and three from Maui, representing grades K, 2, 6 – 8, and 10 -12, completed workshops and activies. Teachers reached 652 students and educated 86 teachers and staff and approximately 900 community members including parents. A pre-course survey showed 55% of teachers agreed to a basic understanding of rat lungworm and its impact on Hawai‘i; this increased to 100% post-course. A sixth-grade class was the first to document the arrival of *Parmarion martensis* the semi slug, an important host of the RLW, in the North Kohala District of Hawai‘i Island. This important discovery initiated community awareness and control efforts.

In addition to the for-credit course, one-day workshops held on the main Hawaiian Islands were attended by 106 participants including teachers, community educators, and interested individuals from agencies, non-profits, businesses, and the private sector. Of participants surveyed, 100% responded that the workshop improved their overall understanding of RLWD. The integration of these activities into K-12 classrooms can be useful to increase public awareness of RLWD, prevent disease, enlist community participation in host control efforts, provide teacher professional development, and introduce students to career connections. Teachers and students engaged in leadership roles, applied critical thinking, and worked in teams to address an important health issue for Hawai‘i. The emphasis on food security in

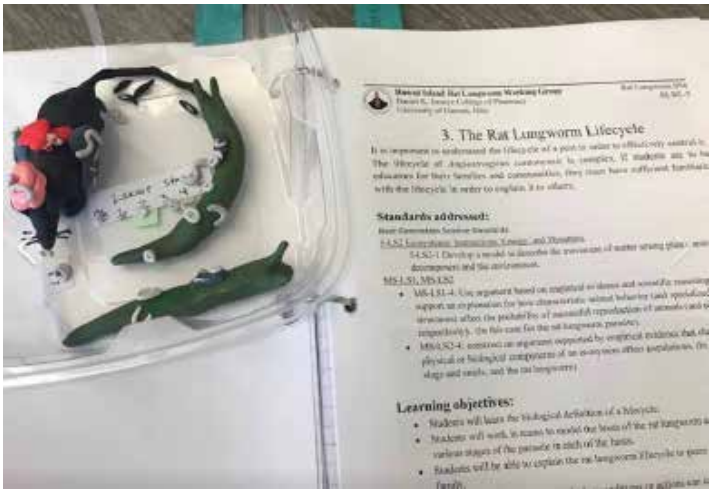

Fig. 1. Hands-on learning helps reinforce understanding of the complex lifecycle of the rat lungworm *Angiostrongylus cantonensis.* Modeling the lifecycle with clay provides a fun and educational activity, reinforcing understanding. Breaking the lifecycle through control of hosts is crucial for disease prevention.

Hawai‘i and the rise in popularity of school gardens provides an excellent opportunity for an educational focus on RLWD.

Original article: Howe, K., Bernal, L., Brewer, F., Millikan, D., & Jarvi, S. (2021). A Hawai‘i public education programme for rat lungworm disease prevention. *Parasitology* **148 (2),** 206-211. doi:10.1017/S0031182020001523

Dr. Argon Steel is a research technician at the Jarvi Lab, Daniel K. Inouye College of Pharmacy, University of Hawai‘i, Hilo. He is currently evaluating commercial and household products for their suitability as produce washes to reduce the threat of infection by *A. cantonensis* the rat lungworm. He has extensive prior experience in natural resource managment, plant ecology, and mosquito control, particularly with the *Aedes* mosquito, a vector of dengue disease.

Comparative studies of treatments and commercially available solutions on mortality of *Angiostrongylus cantonensis* third-stage larvae

Argon Steel[1] , John Jacob[1], Ina Klasner[1], Kathleen Howe[1], Steven H. Jacquier[1], William C. Pitt[2], Robert Hollingsworth[3], Susan I. Jarvi[1*]

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A significant recent increase in rat lungworm infection cases, particularly on Hawai‘i Island, appears to be associated with the accidental ingestion of *Angiostrongylus cantonensis* larvae, primarily in snails or slugs, as well as potentially from larvae left behind in the slug’s slime or feces. With the goal of reducing the risk of rat lungworm contamination of fruits and vegetables, we developed a simple assay to evaluate more than 40 different treatments for their ability to kill *A. cantonensis* infectious-stage larvae. In addition, we investigated the length of time that isolated larvae can survive in the environment, e.g., on counters or benchtops, and under the effects of changing temperature and moisture level. Larval mortality was evaluated by two methods; using larval movement as an indicator of survival after treatment and by applying propidium iodide, a stain that fluoresces in response to lethal cell damage in the larvae. Treatments tested included common household products, consumer vegetable washes, and agricultural crop washes.

Fig.1 Accidental ingestion of rat lungworm through the eating of fruits and vegetables is thought to be a major cause of angiostrongyliasis in Hawai‘i. The semi-slug in the middle of the salad shown, illustrates the challenges in keeping produce safe for consumption.

We found minimal effectiveness from using consumer-grade fruit and vegetable washes, botanical extracts such as ginger or garlic, or acid solutions such as vinegar. Treatments that did show promise in killing *A. cantonensis* included crop washes of high alkalinity, as well as oxidizers such as bleach and chlorine dioxide. Surfactants, a frequent ingredient in detergents that lowers surface tension, had variable results but did seem to increase the efficacy of alkaline solutions. Salt was effective but only when used at high concentrations (15%) for a minimum of 24 hours. We were also interested in investigating other means of reducing infection from *A. cantonensis*, such as contact with household surfaces. Experiments in drying *A. cantonensis* larvae, for example, revealed that few survived beyond 3 minutes of drying on hard, impermeable surfaces such as tabletops and that treating surfaces

with isopropyl alcohol or ethanol further increased larval mortality. Finally, we found that refrigeration had little effect, allowing large numbers of larvae to survive for up to a week, while freezing at -15° C for 12 and 24 hours resulted in high larval death rates. Nevertheless, even with freezing, there was a 2-day delay before achieving close to 100% mortality. These results demonstrate that while close inspection and thorough washing of produce continues to be the most critical preventions against rat lungworm infection, there are treatments that appear to hold promise for further development.

Original article: Steel, A., Jacob, J., Klasner, I., Howe, K., Jacquier, S., Pitt, W., Hollingsworth, R., & Jarvi, S. (2021). *In vitro* comparison of treatments and commercially available solutions on mortality of *Angiostrongylus cantonensis* third-stage larvae. *Parasitology* **148 (2),** 212-220. doi:10.1017/S0031182020001730

Bernard (Chad) Meyer is a consulting physician for Community and Tropical medicine on Maui, Hawai‘i, and is a member of the Clinical Subcommittee of the Hawai‘i Governor’s Task Force on Rat Lungworm Disease. He received his MSc at the London School of Hygiene and Tropical Medicine at the University of London, and his MD from the Medical College of Georgia, USA.

Chronic neuroangiostrongyliasis (NAS, rat lungworm): case study of chronic presentations in Hawai’i

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A study group of 10 confirmed neuroangiostrongyliasis (NAS) cases was recruited from persons infected and diagnosed in Hawai’i. During the interval 2014–2017 the State of Hawai’i Department of Health recorded 38 confirmed cases. Although the present study was able to include only 7 of these 38 cases, they represent chronic disabilities in a minimum 18% (7 of 38) of the reported cases. Additional anecdotal cases with similar chronic characteristics have been reported in patient support groups but have not been included because of inability to access medical records.

Clinical presentations

This study demonstrated that Angiostrongyliasis in Hawai’i is frequently life changing. Many persons sustain residual symptoms for years, including troublesome sensory hyperesthesia (burning and needle point skin irritation), numbness, extremity muscle pains; employment and economic hardships; sleep disorders, psychological impairments and domestic relocations. The skin hyperesthesia resembles chronic zoster neuralgia (shingles), and its intensity (and search for remission) is reflected in an extensive list of attempted therapeutics. Two persons experienced balance deficits limiting activities, sometimes associated with falls. Emotional depression was frequent and economic problems resulting from prolonged work absence and disabilities were common. Several persons noted prolonged caregiver dependency. Pre-existing conditions were complicated by NAS infection. Two females experienced urinary retention presumably from sacral plexus nerve damage; one had related ascending urinary tract infections ultimately resulting in renal abscess and sepsis. Patients universally expressed frustration at the lack of health care recognition for both acute and chronic components of the illness, and how this contributed to a sense of therapeutic inadequacy bordering on a sense of health care provider indifference.

Environmental issues influencing infection parasite dose and clinical outcomes, and geographic differences in disease presentation

Case reports from Hawai‘i suggest a disease spectrum different from Southeast Asia and China. Severe acute infections with chronic symptoms are frequently noted in Hawai‘i but are rarely reported from Southeast Asia and China. The paper hypothesizes mollusc species (snails and slugs) bearing high parasite loads produce more severe disease. In general, aquatic mollusks (rice fields) are found to have relatively lower parasite loads than terrestrial mollusks because of less frequent encounters with rats and rat feces. Locations, where terrestrial mollusc species exist in frequent and prolonged contact with rats, promote the development of snail and slug populations characterized by high parasite loads.

Infections in SE Asia and Southern China result from aquatic Pomacea and Pila species snails eaten as food sources that have been inadequately heated prior to consumption. In contrast, infections in Hawai’i predominantly result from inadvertent, and often unrecognized, mollusk exposures. Hawai’i mollusc surveys have found parasite

prevalence and load in terrestrial molluscs to be higher than what is commonly observed in aquatic snail species in Southeast Asia and southern China. This suggests in Hawai‘i there is a higher risk of ingesting a large number of viable L3 larvae, and corresponds with the reported intensity and duration of cases in the present study.

In Hawai’i, recent surveys have found 16 terrestrial species that are carriers of *A. cantonensis*. Although any of the 16 is capable of transmission, the ‘semi-slug’ *Parmarion martensi* is frequently implicated as a major contributor to outbreaks of severe disease in Hawai’i. *Veronicella cubensis*, “Cuban slug”, exhibited an infection prevalence of only 3–4%; however, because it is so frequently found in home garden produce it may also pose an important risk for transmission.

Original article: Meyer, B. (2021). Chronic neuroangiostrongyliasis: Case study of chronic presentations in Hawai‘i. *Parasitology* **148 (2),** 221-226. doi:10.1017/S0031182020001651

Dr. Vernon Ansdell is an Associate Clinical Professor in the Department of Tropical Medicine, Medical Microbiology and Pharmacology at the John A. Burns School of Medicine at the University of Hawai, Manoa. He practiced and taught at the London School of Tropical Medicine and Hygiene and the Hospital for Tropical Diseases in London. He has an interest in tropical disease, with a particular interest in leptospirosis, seafood poisonings, marine envenomations and neuroagniostrgyliasis. He is currently working on national, evidence-based guidelines for the diagnosis and treatment of neuroangiostrongyliasis.

Updated recommendations for the diagnosis and treatment of neuroangiostrongyliasis (rat lungworm disease).

Vernon Ansdell[1], Kenton J. Kramer[1], Jourdan K. McMillan[1], William L. Gosnell[1], Gerald S. Murphy[1], B. C. Meyer[2], Elizabeth U. Blalock[3], Johnnie Yates[4], Louis Lteif[5], Olivia A. Smith[1] and Marian Melish[6]

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In 2018, a clinical subcommittee of the Hawai'i Governor's Joint Taskforce on Rat Lungworm Disease (RLWD) in Hawai'i developed preliminary guidelines for diagnosing and treating neuroangiostrongyliasis (NAS). These preliminary guidelines were updated following an extensive review of the literature and were published in a recent supplement of the journal Parasitology (Oxford). Important recommendations include:

Lumbar puncture (LP or spinal tap) is a low-risk procedure and is an essential part of evaluating suspected rat lungworm disease (RLWD). It enables examination of cerebrospinal fluid (CSF), which is necessary to diagnose RLWD and exclude other causes for the patient's symptoms. Removal of the CSF may also provide relief from headaches.

Polymerase chain reaction (PCR) of CSF for *Angiostrongylus cantonensis* DNA is currently the best way to confirm the diagnosis of RLWD.

The threshold for testing cerebrospinal fluid (CSF) with PCR should be i) a strong exposure history and ii) convincing clinical physical symptoms and signs. Eosinophils in the CSF are usually present in cases of RLWD but not essential before performing PCR testing if the other elements are present.

Physicians should start treatment with steroids and albendazole as soon as a presumptive diagnosis is made. There is no need to wait for the results of PCR testing before beginning treatment.

Conclusive diagnosis of RLWD (NAS) requires a positive PCR (polymerase chain reaction) for *Angiostrongylus cantonensis.* If there is a strong suspicion of RLWD, early treatment should be started before obtaining confirmatory results. If the PCR result is negative, and the diagnosis is still suspected, the LP and PCR should be repeated.

Early diagnosis and treatment with steroids and albendazole appear to be very important and may help prevent some of the serious long-term effects of this disease.

Steroids should be given with albendazole (to kill the parasite) to limit the possibility of a severe inflammatory reaction to dead and dying worms.

Patients require careful clinical monitoring during and after treatment to respond promptly to any complications.

Collaboration and research with RLWD centers around the world will be necessary to answer the many remaining questions regarding this important and challenging disease. Important areas for study include developing sensitive serologic (blood) tests and gathering more evidence to support treatment options for individuals with long term (chronic RLWD) complications.

Copies of "Guidelines for the diagnosis and treatment of neuroangiostrongyliasis: updated recommendations" and "Preliminary Guidelines for the Diagnosis of Treatment of Neuroangiostrongyliasis (Rat Lungworm Disease) in Hawai'i" can be accessed by scanning the accompanying QR code with a smartphone.

Parasitology

Preliminary

Original article: Ansdell, V., Kramer, K., McMillan, J., Gosnell, W., Murphy, G., Meyer, B., Blalock, E.U., Yates, J., Lteif, L., Smith, O.A., & Melish, M. (2021). Guidelines for the diagnosis and treatment of neuroangiostrongyliasis: Updated recommendations. *Parasitology* **148 (2),** 227-233. doi:10.1017/S0031182020001262

Dr. Praphathip Eamsobhana is active in teaching and scientific research with the Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University in Bangkok, Thailand. She is recognized internationally for her research publications on rat lungworm disease and laboratory diagnostics. She was one of the pioneers to adopt a holistic and innovative approach in immunodiagnosis for eosinophilic meningitis caused by the rat lungworm. Her noteworthy achievment and contribution is the finding of a 31 kDa glycoprotein antigen of *A. cantonensis* for the rapid diagnosis of human rat lungworm disease.

Detection of *Angiostrongylus cantonensis* specific antigens in serum for diagnosis of active neuroangiostrongyliasis

Praphathip Eamsobhana[1*], Anchalee Tungtrongchitr[1*], Hoi-Sen Yong[2], Anchana Prasartvit[3], Darawan Wanachiwanawin[1], Xiao-Xian Gan[4]

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Suspected cases of *A. cantonensis* infection can only be confirmed by isolation of the nematodes in cerebrospinal fluid (CSF) or eye chamber of the infected individuals, but this finding is rare. Presumptive diagnosis is primarily based on clinical characters, specific eating habits, peripheral blood eosinophilia and serological testing (diagnostic methods that are used to identify antibodies and antigens in a patient's sample). Currently, the immunological diagnosis of human infection to detect specific *A. cantonensis* antibodies/antigens remains problematic because there are no commercially available validated tests. Development of a more reliable, user-friendly test to support clinical diagnosis of eosinophilic meningitis/meningoencephalitis due to *A. cantonensis* is still needed.

Serological tests may yield false-negative results for specific antibodies detection before or at early seroconversion phase, when a person develops antibodies to the disease-causing microorganisms/pathogens. Tests that detect circulating antigens of *A. cantonensis* would be of value to distinguish current or past infection. We developed a quick, easy to perform, portable and inexpensive diagnostic device for detection of 31-kDa *A. cantonensis* specific antigens. The sandwich dot-immunogold filtration assay (*Ac*DIGFA^{Ag}), for detecting active angiostrongyliasis was produced using anti-*A. cantonensis* polyclonal antibody dotted on nitrocellulose membrane as a capture agent and colloidal gold-labeled anti-31 kDa *A. cantonensis* antibody as a detection agent. A well-defined pink dot, indicating positivity, was seen readily by naked eye within 10-15 min. Diagnostic accuracy of *Ac*DIGFA^{-Ag} was evaluated using CSF samples from clinically diagnosed patients with detectable *A cantonensis*-specific antibodies (n = 10), patients with clinically suspected cases that tested negative for *A. cantonensis*-antibodies (n = 5), cerebral gnathostomiasis caused by several species of parasitic nematodes in the genus *Gnathostoma* (n = 2) and neurocysticercosis caused by the presence of cysts of a tapeworm in the brain (n = 3). The *Ac*DIGFA^{Ag} detected *A. cantonensis* specific antigens in CSF samples from four of 10 serologically confirmed angiostrongyliasis cases and two of 5 suspected cases with negative anti-*A. cantonensis* antibodies. No positive *Ac*DIGFA^{Ag} reaction was observed for the remaining CSF samples. Diagnostic potential of *Ac*DIGFA^{Ag} to detect *A. cantonensis* antigen was additionally assayed with serum samples from clinically diagnosed patients with detectable *A. cantonensis*-specific antibodies (n = 19) as well as serum samples from patients with nine other parasitic diseases (n = 43) and 35 samples from normal healthy subjects. Among the 19 sera with *A. cantonensis* infection, two showed positive reaction by *Ac*DIGFA^{Ag}. No positive *Ac*DIGFA^{Ag} reaction was observed in all the serum samples with other parasitic diseases or the healthy

Figure 1. An example of a *Ac*DIGFAAg Test with positive and negative results.

controls. The present vertical flow-through "*Ac*DIGFA^{Ag}" enables rapid qualitative detection of the specific 31-kDa antigens of *A. cantonensis* in clinical samples without complicated steps, showing potential for application in rapid diagnosis of early/active *A. cantonensis* infection, even under resource-limited settings.

Original article: Eamsobhana, P., Tungtrongchitr, A., Yong, H., Prasartvit, A., Wanachiwanawin, D., & Gan, X. (2021). Sandwich dot-immunogold filtration assay (DIGFA) for specific immunodiagnosis of active neuroangiostrongyliasis. *Parasitology* **148 (2),** 234-239. doi:10.1017/S0031182020001894

John Jacob PharmD is currently pursuing his PhD from the University of Hawai‘i, Hilo with Dr. Susan Jarvi. His focus is on the treatment of neuroangiostrongyliasis using anthelmintics. The use of these drugs for the treatment and management of the disease remains controversial due to inconsistent and discrepant conclusions from multiple studies, most of which are case reports and uncontrolled studies. To determine the efficacy of anthelmintics on *A. cantonensis,* John Jacob studies include *in vitro* and *in vivo* investigation of FDA approved anthelmintics on *A. cantonensis.*

Effect of anti-parasitic drugs on rat lungworm L3 larvae

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Rat lungworm parasite *Angiostrongylus cantonensis* is the most common cause of eosinophilic meningitis worldwide, with life-threatening complications if not managed correctly. Previous studies were conducted directly on adult female worms and utilized the change in movement to determine whether an anthelmintic (worm medication) had any effect. However, it is the third stage larvae (L3s), not the adults that are infectious to humans. Propidium iodide is a fluorescent stain that only stains dead cells (indicator of death). Using this stain, we are able to distinguish between dead and live larvae. We used this stain to measure the killing effects of nine clinically established anthelmintics directly on rat lungworm L3 larvae. All of these drugs were tested at a 1 mM (a high concentration). Piperazine and niclosamide did not show any effect, however, albendazole, pyrantel pamoate (pinworm medication), diethylcarbamazine, levamisole, and praziquantel showed significant mortality on L3 *A.*

Anthelmintic (1 mM)	Putative MOA on L3	Behavioral changes within 60 min PE	Day PE of death	Mean % death by day 30 PE	
				Treatment	Control
Albendazole sulphoxide	Inhibits microtubule assembly	None	15	80.38 ± 10.62 SD	32.75 ± 5.89 SD
Diethylcarbamazine	Unknown	None	11	82.4 ± 7.12 SD	28.25 ± 15.20 SD
Levamisole Hydrochloride	Nicotinic agonist	Tightly coiled	15	97.25 ± 3.57 SD	6.25 ± 1.8 SD
Pyrantel pamoate	Nicotinic agonist	Sluggish and/or coiled	12	94 ± 2.16 SD	41.5 ± 12.02 SD
Pyrantel citrate	Nicotinic agonist	Tightly coiled	3	100 ± 0 SD	-----
Praziquantel	Calcium influx induced paralysis	None	22	50.8 ± 2.77 SD	41 ± 2.83 SD
Niclosamide ethanolamine	Unknown	None	----	10 ± 3.29 SD	34.75 ± 7.42 SD
Piperazine	Partial GABA† agonist	None	----	4.1 ± 2.02 SD	2.5 ± 0.71 SD
Ivermectin-TPGS*	GABA† agonist	Sluggish	----	2.62 ± 1.18 SD	8.75 ± 2.75 SD
Moxidectin-TPGS*	GABA† agonist	Sluggish	----	1.5 ± 1.06 SD	5.75 ± 3.4 SD

Table 1 Anthelmintic drugs tested, putative mechanism of action (MOA) on L3, observed behavioral changes within 60 mins post-exposure (PE), and the mean percent larval death 30 days PE with 1 mM drug concentration.

* TPGS (2%w/v) alone was used as controls.

†GABA, Gamma-aminobutyric acid.

cantonensis. Ivermectin and moxidectin did not show any killing effect, but they considerably reduced larval movement almost immediately (Table 1). Although some of these anthelmintics resulted in mortality of L3 on the lab bench, we will next conduct animal trials to determine the correct dose and treatment duration after exposure to L3 *A. cantonensis*, starting with the pinworm medicine pyrantel pamoate, to have a better understanding of their potential effects during human infections.

Original article: Jacob, J., Tan, G., Lange, I., Saeed, H., Date, A., & Jarvi, S. (2021). *In vitro* efficacy of anthelmintics on *Angiostrongylus cantonensis* L3 larvae. *Parasitology* **148 (2),** 240-250. doi:10.1017/S0031182020001146

Elizabeth Atkinson completed this study as a science project while attending Hilo High School. She won numerous science fair awards at the local level, allowing her to participate at the state and international levels. She is currently a Biology major at Swarthmore College in PA, with interests in veterinary medicine.

Recombinase polymerase amplification (RPO-EXO) and lateral flow assay (RPA-LFA) detect *Angiostrongylus cantonensis* DNA in intermediate gastropod hosts.

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Currently, a laboratory test called qPCR (quantitative polymerase chain reaction) is one of the most reliable diagnostic methods available for detecting *Angiostrongylus cantonensis* DNA in humans as well as in gastropod hosts, but it requires expensive and specialized equipment, takes 1-2 hours to run a sample, and trained professionals are needed to operate and interpret results. The benefits of qPCR are more applicable to large scientific studies, where qPCR offers the ability for high throughput and automated systems to process many samples at once as well as the ability to quantify exact DNA concentrations in a sample. Field and clinical settings generally process fewer samples at a time and do not require quantitative analysis, thus, qPCR is not necessary, and other available technology could offer options better suited for these non-laboratory environments. Recombinase polymerase amplification (RPA) is a different type of laboratory test that uses less expensive equipment, takes 10-15 minutes to run a sample, and the results can be interpreted without extensive training. RPA is more adaptable to small lab spaces that do not need to test large numbers of samples. Lateral flow is a third type of laboratory test that, along with RPA, can further minimize cost and time requirements for testing tissue samples for the presence of rat lungworm DNA. Lateral flow test strips allow the results to be determined visually by the presence of two bands, similar to a pregnancy test. In this study, a new assay was developed using RPA and lateral flow techniques to detect the presence of rat lungworm DNA in slug tissue samples.

This new RPA-Lateral Flow assay was developed by using software to visualize the DNA sequence of rat lungworm and to create smaller strings of nucleotides called primers and probes that allow the rat lungworm DNA to be amplified and detected using RPA techniques. Different combinations of primers and probes were tested to determine which two primers and which probe would most effectively replicate the rat lungworm DNA and thus have the most reliable results. The final assay (consisting of the set of primers and probe) was then tested on samples of DNA extracted from slug tissue collected from east Hawai’i Island to determine if the tissue samples contained rat lungworm DNA and thus were infected with the parasite. This RPA assay was then adapted to be used with lateral flow strips and the same samples were tested using the RPA-lateral flow assay. As a baseline for comparison, the same samples were then tested using the qPCR assay that is currently being used to detect rat lungworm in slugs and the results were compared to the results of the new RPA and RPA-lateral flow assays. Both the accuracy (number of samples that had the same result for both tests) and the sensitivity (determined by testing a series of different dilutions of rat lungworm DNA copies) of the three tests were tested in this study.

The three assays were used to test 35 slugs from Hawai‘i for the presence of *A. cantonensis* DNA. Consistent results among the three tests were shown in 23/35 samples (65.7%), while 7/35 (20%) did not have consistent results (but had very low infection level of <0.01 larvae per mg tissue), and 5/35 (14.3%) did not have definitive results. To evaluate sensitivity, a section of the rat lungworm DNA was cloned, and a plasmid was created. Dilutions of this

plasmid were created ranging from 100 copies per test to ~1 copy per test. All three assays consistently detected 50–100 copies per test in three replicates and qPCR was able to detect ~13 copies per test in three replicates. The RPA assay was able to detect 25 copies per test in three replicates and RPA-lateral flow assay was not able to consistently detect below 50 copies per test in three replicates (however, both assays detected ~13 copies per test in 2 of 3 replicates). Thus, our RPA and RPA-lateral flow assays do not appear as quite as sensitive as the current qPCR assay at low DNA concentrations; however, these new assays are reliable at higher rat lungworm infection levels (higher DNA levels in tissue samples) and have numerous advantages that may make them useful alternatives to qPCR. Examples of lateral flow assay test results are shown in Figure 1.

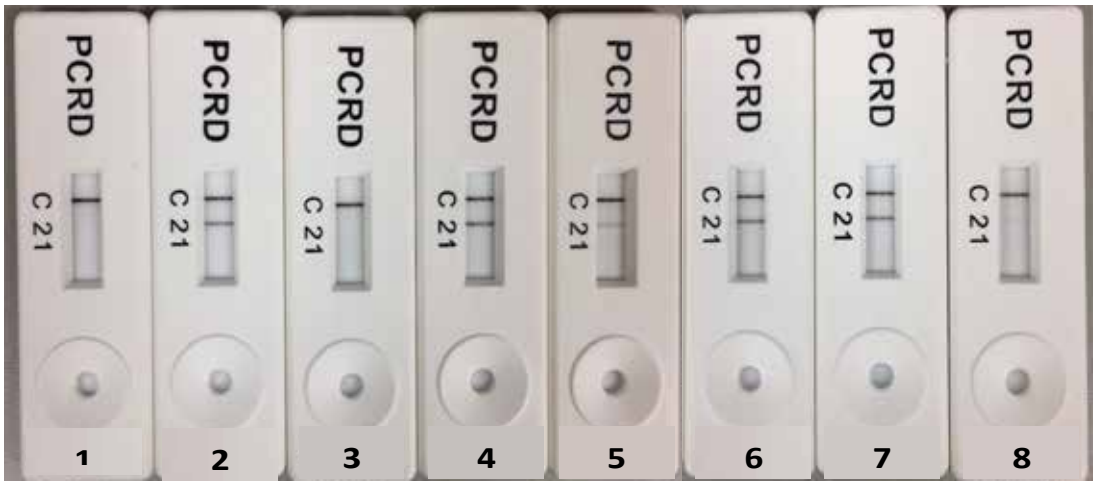

Figure 1.RPA-LFA test results show negatives as one control band (Nos. 1 and 3), positives as two bands (Nos. 2, 4, 6, 7), and faint positives indicating very low levels of DNA present (Nos. 5 and 8).

Both the RPA and RPA-lateral flow assays could become low-cost alternatives to qPCR for monitoring of rat lungworm prevalence in Hawai'i for researchers, clinicians, and homeowners alike. Although these new assays may not quite as sensitive as qPCR, with further development they could be used to decrease the cost and time needed for human and veterinary diagnostics. In situations where a rapid, inexpensive detection method is needed for rat lungworm, RPA and RPA-lateral flow tests provide reliable alternatives to qPCR with samples expected to have higher DNA concentrations.

Original article: Jarvi, S., Atkinson, E., Kaluna, L., Snook, K., & Steel, A. (2021). Development of a recombinase polymerase amplification (RPA-EXO) and lateral flow assay (RPA-LFA) based on the ITS1 gene for the detection of *Angiostrongylus cantonensis* in gastropod intermediate hosts. *Parasitology* **148 (2)**, 251-258. doi:10.1017/S0031182020002139

Presenters and survivors of rat lungworm disease attending and participating in the Workshop.

Back Row, left to right:

Hung-Chin Tsai MD PhD, Suzy Papanikolas, Praphathip Eamsobhana PhD, Gerald Murphy MD. Bernard Meyers MD, Robert Cowie PhD, Kittisak Sawanyawisuth MD PhD, Lorrin Pang MD. Argon Steel PhD, Lindsey Hamilton MS, Kirsten Snook MS, Richard Malik DVS, Sarah Strong ND, Jon Martell MD, Patrick Sullivan, Kornelia DeKorne.

Second row from back, middle to right:

Jenee Odani DVM, Yaeko Tagami, Susan Jarvi PhD., Darlene Turner, Ann Szaur RN MS, Shawzy Cann

Front Row:

Vernon Andsell MD, Heather D S Walden PhD, Chris Niebuhr PhD, Kathleen Howe MS, Randi Rollins MS, David Modrý PhD DVM, Rogan Lee PhD, John Jacob PharmD, Lisa Kaluna MS (behind kneeling), Barbara Fecková PhD, Esperanza Hilton RN (seated below) Matthew Wun DMV, Jan Šlapeta PhD MDV

Presenters missing from photo:

Noppadol Aekphachaiswat PhD, Mark LeRoy & Maya Parish, Shan Lv, Alfred J. Mina DVM, Lorrin Pang MD, MPH

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