USDA APHIS Wildlife Services
National Wildlife Research Center
Hawaii Field Station

Alternative Hosts of Rat Lungworm FY18 Final Report

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Report to Hawaii Invasive Species Council

19 July 2019

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Introduction

Rat lungworm (*Angiostrongylus cantonensis*) is an emerging zoonotic pathogen that causes rat lungworm disease (angiostrongyliasis) in humans worldwide, including Hawai‘i. Human exposure can result from the ingestion of contaminated fruits and vegetables, and concerns exist about the risk to water catchment systems (Hollingsworth et al. 2007, Ewers and Anisowicz 2014). This parasite not only threatens people living in Hawai‘i, but is also a health concern for those who may be visiting. In particular, eastern Hawai‘i Island has recently reported infection rates in humans, slugs, and rats to be higher than anywhere else in the U.S. (see Jarvi et al. 2017).

The lifecycle of the rat lungworm is complex, requiring both intermediate hosts and definitive hosts, but can also involve paratenic (transport) hosts (Wang et al. 2012). Known rat lungworm hosts in Hawai‘i are: mollusks (snails and slugs) as intermediate hosts, rats (*Rattus* spp.) as definitive hosts, and planarians (flatworms) as paratenic hosts. However worldwide, many other animal species have been identified as hosts of rat lungworm, and thus the current list for Hawai‘i may be incomplete. For example, crabs, frogs, and lizards, have been identified as paratenic hosts in other countries, with one study identifying 53% of introduced frogs to the island country of New Caledonia to contain infective larvae (Wang et al. 2008, Ash 1968). Additionally, rat lungworm has been reported in a variety of “accidental hosts,” such as dogs, horses, sheep, bats, and multiple bird species (Spratt 2015), sometimes resulting in debilitating negative health consequences or death; however little is known of the role of these hosts in the maintenance or transmission of the parasite in the wild. The lack of investigation into alternate hosts of rat lungworm in Hawai‘i highlights a gap in the understanding of local transmission cycles. This study is one of the first to address these gaps, especially regarding wildlife.

Gaps in knowledge also exist for those species known to host rat lungworm in Hawai‘i. In collaboration with the University of Hawaii at Hilo Daniel K. Inouye School of Pharmacy (UH Hilo), USDA WS-NWRC has already begun investigating factors influencing lungworm infection levels in wild rat populations, such as differences between species and body mass, some of which has already been published (see Jarvi et al. 2017); however, the influence of season on infection levels in rats has not been investigated. While this type of research is being conducted elsewhere in the world, such as China (Chen et al. 2011), Brazil (Simões et al. 2014), and Australia (Aghazadeh et al. 2015), no such studies exist in Hawai‘i, the greatest hotspot for rat lungworm disease in the United States.

This work builds on previous rat lungworm studies conducted by USDA WS-NWRC in collaboration with UH Hilo: QA-1998 (resulting in Jarvi et al. 2015); QA-2747 (resulting in Jarvi et al. 2017).

Objectives

The overall focus of this on-going research is to further our understanding of patterns of infection of rat lungworm in wildlife hosts in Hawai‘i. Here we focus on two objectives to help fill the current gaps in knowledge.

1. To identify potential alternate wildlife host candidates for the presence of rat lungworm

2. To investigate the seasonality of rat lungworm infection in wildlife hosts

Methods

Study sites

Sampling took place in two locations (Figure 1), both of which are areas of known rat lungworm infections in wild rat populations (Jarvi et al. 2017).

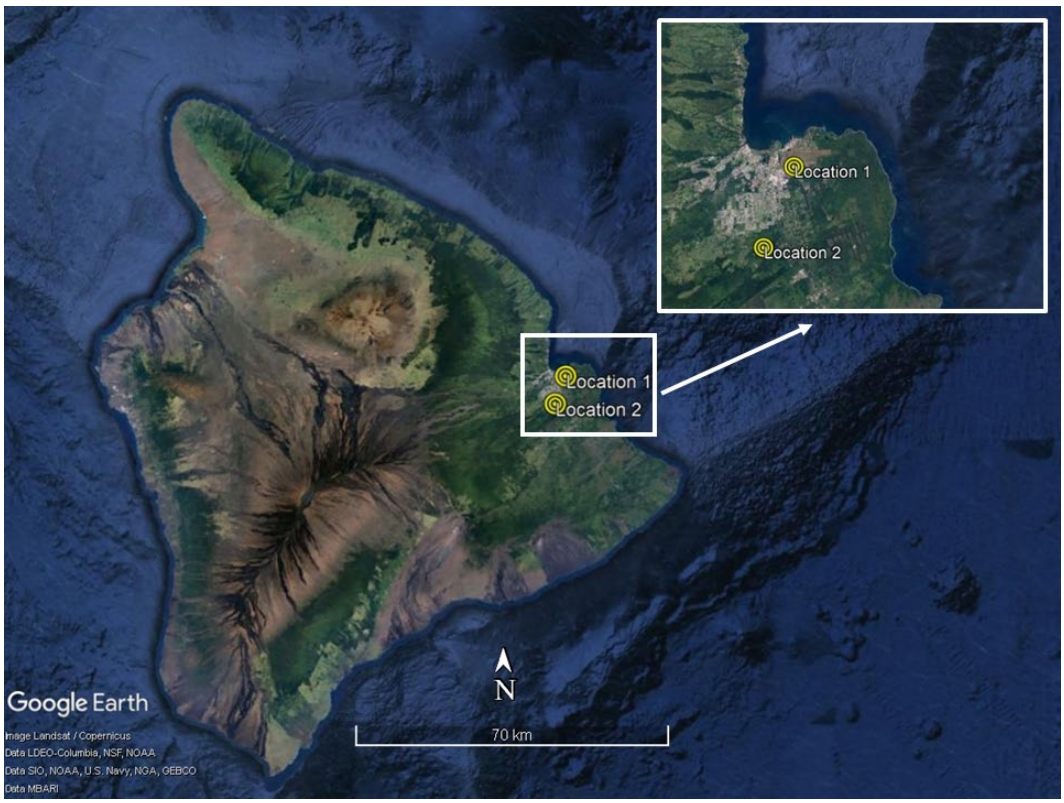

Figure 1. *Rat lungworm sampling locations on eastern Hawai'i Island.*

Wildlife hosts - morphological analysis

To investigate the potential for wildlife species to act as alternate hosts of rat lungworm, we sampled two invasive vertebrate species, the small Indian mongoose (*Herpestes auropunctatus*) and the common myna (*Acridotheres tristis*). Both mongooses and myna are generalists, commonly found on Hawai'i Island, including Location 1. Mongooses used in this study were caught in the rat traps from Location 1 and processed similarly to the rats (see 'seasonality of infections' section). Myna carcasses were provided by Wildlife Services during the course of their nuisance animal removal duties in and around Location 1, and were provided frozen. Carcasses were dissected and the heart/lung complex and brain were inspected for adult and sub-adult stages of rat lungworm.

Wildlife hosts - molecular analysis

To identify the presence of rat lungworm larvae in wildlife hosts, tissue samples were taken of a variety of wildlife species. Liver, heart/lung, and brain samples were collected from 20 mongooses and muscle, heart/liver/lung, and brain samples from 20 myna. These were collected from the same individuals used for the morphological inspections of adult and sub-adult worms. We sampled 24 *E. coqui* on 21 June 2018 from a site in Hilo. In addition, four greenhouse frogs (*Eleutherodactylus planirostris*), two cane toads (*Rhinella marina*, and three Chinese red-headed centipedes (*Scolopendra subspinipes*) were sampled. These samples were opportunistically collected between February and June 2018, all within 20 km of the *E. coqui* collection site. Tissue samples from frogs and toads comprised five distinct regions, including digestive tract, muscle, liver, heart, and brain. The centipede samples consisted of scrapings of internal organs from within the exoskeleton. Real-time PCR was carried out to identify the presence of *A. cantonensis*, as previously described in Jarvi et al. (2012).

Seasonality of infections

To assess the seasonality of infection levels of rat lungworm in wild rat populations, rats (*Rattus rattus* and *R. exulans*) were sampled on four separate occasions (May, August, November 2018, and February 2019). Rats were live-trapped from two locations on the eastern side of Hawai‘i Island via standard wire-cage live traps and transported back to the USDA WS-NWRC Hawai‘i Field Station according to standard NWRC protocols. There, rats were humanely euthanized and dissections were conducted to identify the presence of adult rat lungworms. Worms collected from the lungs and pulmonary arteries were identified and counted.

Additionally, 20 semi-slugs (*Parmarion martensi*) were collected from each location and seasons as the rat trapping occurred. Slugs were collected by hand (using gloves; Hollingsworth et al. 2013), stored in a sealed plastic bag, and then frozen (-80°C). These samples were then sent to UH-Hilo for PCR analysis to asses the presence of rat lungworm larvae.

Results

Wildlife hosts (morphological analysis to identify presence of adult stages)

All mongooses and myna in this study were found to be negative for adult and sub-adult worms by morphological inspection (Table 1).

Table 1. *Results of morphological inspection for adult and sub-adult stages of rat lungworm (Angiostrongylus cantonensis) in wildlife from eastern Hawai‘i Island.*

Species	Common name	Sample size	No. positive	
			Heart/lungs	Brain
Herpestes auropunctatus	Small Indian mongoose	20	0	0
Acridotheres tristis	Common myna	20	0	0

Wildlife hosts (molecular analysis to identify presence of larvae stages)

Of the 24 *E. coqui* sampled, 21 (87.5%) were identified as positive for *A. cantonensis* via real-time PCR in at least one tissue type per individual (Table 2). Overall, parasite presence was detected in each of the five tissue types sampled (stomach/intestine, muscle, liver, heart, and brain), with 14 frogs positive for at least three different tissue types. Of note, a whole semi-slug (*Parmarion martensi*) was found in the stomach of a 4.50 g *E. coqui* that had positive heart, liver, muscle, and stomach/intestine samples. The slug also tested positive for *A. cantonensis* via PCR. The invasive *P. martensi* is capable of carrying heavy parasite burdens and has been identified as a highly competent intermediate host of *A. cantonensis* in Hawai‘i (Hollingsworth et al. 2007). In addition, the four *E. planirostris*, two *R. marina*, and three centipedes were all positive for *A. cantonensis* (Table 2). Detailed results from each tissue type, as well as an expanded discussion, can be found in Niebuhr et al. (in press), also included as Appendix A.

Table 2. *Results of real-time PCR detection of rat lungworm (Angiostrongylus cantonensis) in invasive hosts from eastern Hawai‘i Island. TBD = to be determined.*

Species	Common name	Sample size	No. positive
Eleutherodactylus coqui	Puerto Rican coqui frog	24	21
Eleutherodactylus planirostris	Cuban greenhouse frog	4	4
Rhinella marina	Cane toad	2	2
Scolopendra subspinipes	Chinese red-headed centipede	3	3
Herpestes auropunctatus	Small Indian mongoose	20	TBD
Acridotheres tristis	Common myna	20	TBD

Seasonality of infections

Results here only include data from the first three seasons (dissection data from February 2019 is still being collected). A total of 317 rats were sampled for rat lungworm in the first

three seasons. Observed prevalence (presence/absence) of adult rat lungworms in rats sampled can be seen in Table 3. Observed prevalence varied by season for both species (Figure 2). Of note, the observed prevalence of within *R. exulans* in May was significantly higher than any other seasons, including observations in *R. rattus*, in which all rats sampled (n=64) were infected. For *R. exulans*, a similar decreasing trend by season was seen for average number of worms as for observed prevalence (Table 4). In our study, we found the overall observed prevalence to be higher in *R. exulans* (70.4%; 119/169) than *R. rattus* (47.3%; 70/148). A similar trend by species was previously observed in February 2017 (Jarvi et al. 2017). The overall observed prevalence of rat lungworm in *R. exulans* varied by location, with Location 1 (78.8%; 78/99) significantly higher than Location 2 (58.6%; 41/70) ($P < 0.05$, two-tailed Fisher's exact test). No difference was observed in *R. rattus* between Location 1 (42.6%; 43/101) and Location 2 (57.4%; 27/47) ($P > 0.05$, two-tailed Fisher's exact test).

Table 3. *Observed prevalence of adult rat lungworm (Angiostrongylus cantonensis) from rat definitive hosts (Rattus exulans and R. rattus) from eastern Hawai'i Island. Sampling took place during four months, May, August, November 2018, and February 2019, with samples from the latter still being processed. TBD = to be determined.*

	R. exulans	*R. rattus*	Total
May-18	100% (64/64)	48.3% (29/60)	75.0% (93/124)
Aug-18	61.9% (26/42)	25.9% (7/27)	47.8% (33/69)
Nov-18	46.0% (29/63)	55.7% (34/61)	50.8% (63/124)
Feb-19	TBD	TBD	TBD

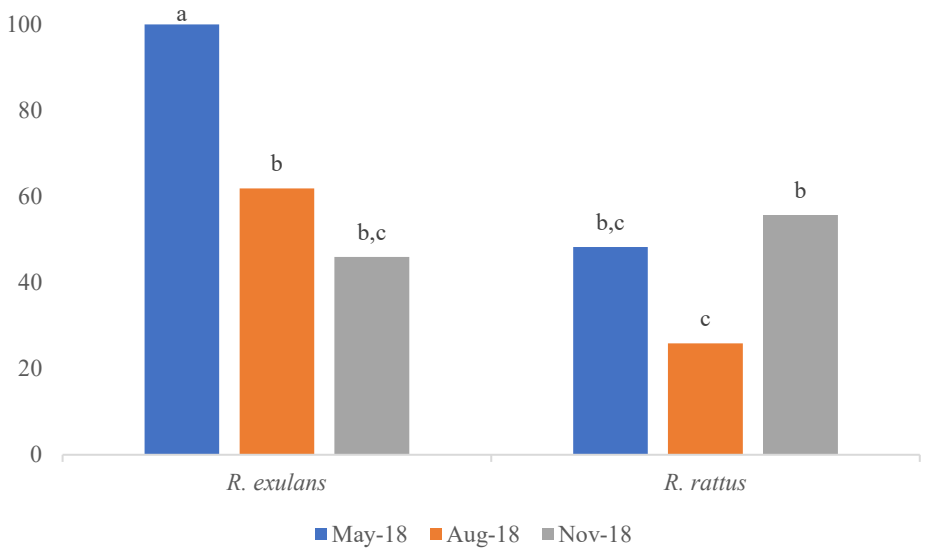

Figure 2. *Observed prevalence of adult rat lungworm (Angiostrongylus cantonensis) from rat definitive hosts (Rattus exulans and R. rattus) from eastern Hawai'i Island. Results presented are from samples collected during three months, May, August, and November 2018. Columns with different letters differ (P < 0.05, two-tailed Fisher's exact test).*

Table 4. *Average number of adult rat lungworms (Angiostrongylus cantonensis) observed in the lungs of infected wild rats (Rattus exulans and R. rattus) from eastern Hawai'i Island. Sampling took place during four months, May, August, November 2018, and February 2019, with samples from the latter still being processed. TBD = to be determined.*

	R. exulans	*R. rattus*	Total
May-18	13.8	4.7	11
Aug-18	11.7	6.4	10.5
Nov-18	6.7	5.6	6.1
Feb-19	TBD	TBD	TBD

Discussion

This is the first study to investigate wildlife hosts of rat lungworm in Hawai'i, and results here confirm the presence of infection in some species. We sampled a variety of species in an area identified previously with extremely high infections in rat definitive hosts. Negative morphological results of adult and sub-adult worms in mongooses and myna, suggest they do not play a major role, if any, in transmission of the parasite. The identification of rat lungworm infection in frogs, toads, and centipedes, however, suggest their potential as reservoir hosts, which could have both management and health implications. Further discussion regarding these potential paratenic hosts can been seen in Appendix A.

This study is also the first to investigate seasonal differences in rat lungworm infection in rat populations in Hawai‘i. Differences in infection levels observed may help land managers and members of the public in making decisions on the timing of rat control operations as well as highlight peaks of potential disease transmission to humans, domestic animals, or livestock. Results from this study may also help to develop a standardized protocol to investigate state-wide distribution of rat lungworm and allow for further studies/surveys that look to investigate distribution or changes over time.

Conclusion

This report covers the full portion of the study funded by HISC and a portion of the study funded in-kind by WS-NWRC. Accounting of the expenditures of HISC funds is included in Appendix B. Completed morphological and molecular analyses, as well as results from the fourth season of sampling, will be included in a comprehensive final report to be provided to HISC and submitted for publication in a peer-reviewed journal.

Acknowledgments

We thank the USDA WS-NWRC Hawaii Field Station, including R. Sugihara and T. McAuliffe for assistance with rat trapping and USDA Wildlife Services for providing myna samples. We thank the Jarvi Lab at UH-Hilo, including S. Jarvi, L. Kaluna, B. Torres Fischer, and K. Snook. Additional thanks is necessary for Craig Blaisdell of the State of Hawaii Department of Defense Environmental Office for his enthusiasm and cooperation. All animal use was reviewed and approved by the NWRC Institutional Animal Care and Use Committee under protocol QA-2835. The findings and conclusion in this preliminary report have not been formally disseminated by the U. S. Department of Agriculture and should not be construed to represent any agency determination or policy.

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Appendix A

Niebuhr, C. N., S. I. Jarvi, L. Kaluna, B. L. Torres Fischer, A. R. Deane, I. L. Leinbach, and S. R. Siers. In press. Occurrence of rat lungworm (*Angiostrongylus cantonensis*) in invasive coqui frogs (*Eleutherodactylus coqui*) and other hosts in Hawaii. Journal of Wildlife Diseases. Online ahead of print.

SHORT COMMUNICATIONS

DOI: 10.7589/2018-12-294

Journal of Wildlife Diseases, 56(1), 2020, pp. 000–000

Occurrence of Rat Lungworm (*Angiostrongylus cantonensis*) in Invasive Coqui Frogs (*Eleutherodactylus coqui*) and Other Hosts in Hawaii

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ABSTRACT: The rat lungworm (*Angiostrongylus cantonensis*) has emerged as an important human and animal health concern in Hawaii. Although the life cycle of the parasite requires both rat and gastropod hosts, other animals acting as paratenic hosts, such as frogs and centipedes, have been identified as sources of infection. We investigated the occurrence of rat lungworm infections in potential paratenic hosts in Hawaii to provide information on how they might be involved in transmission of angiostrongyliasis. We confirmed the presence of rat lungworm in 87% (21/24) of introduced Puerto Rican coqui frogs (*Eleutherodactylus coqui*) in Hilo, Hawaii, USA, by real-time PCR. Additionally, four Cuban greenhouse frogs (*Eleutherodactylus planirostris*), two cane toads (*Rhinella marina*), and three centipedes (*Scolopendra subspinipes*) were found to be infected. In the frogs and toads, multiple tissue types were positive, including stomach and intestine, muscle, liver, heart, and brain, indicating larval migration. We identified rat lungworm infections in frogs, toads, and centipedes in Hawaii and highlighted the lack of knowledge of the role paratenic hosts may be playing in the transmission and life cycle maintenance of rat lungworm in Hawaii.

Key words: Cane toad, centipede, frog, Hawai'i, nematode, parasite, paratenic.

The rat lungworm (*Angiostrongylus cantonensis*) is a tropical and subtropical parasitic nematode that causes angiostrongyliasis (rat lungworm disease) in humans and other animals. Infections typically occur as a result of intentional or unintentional ingestion of animals or animal parts that contain infective third-stage parasite larvae. The life cycle of the rat lungworm requires both rat definitive hosts and gastropod intermediate hosts. Additionally, paratenic (transport) hosts can play an important role in transmission, acting as disease reservoirs, but in which no development of the parasite occurs. For example, frogs and centipedes have been identified as a source of human infection by rat lungworm (Cuneo et al. 2006; Wang et al. 2018). One study of an introduced frog species in New Caledonia reported 53% (23/43) of frogs sampled from market gardens to be infected with rat lungworm (Ash 1968). Natural infections of rat lungworm larvae have been found in multiple tissue types of frogs, including muscle, liver, digestive tract, and the heart-lung complex (Ash 1968; Asato et al. 1978). Other paratenic hosts of rat lungworm have also been identified, including some crustaceans, flatworms (planarians), and lizards (Wang et al. 2008).

In Hawaii, a high level of human cases of angiostrongyliasis have recently been reported, as well as high infection levels in both rat and gastropod hosts (Kim et al. 2014; Jarvi et al. 2017, 2018); however, little research has been conducted on paratenic hosts in Hawaii, except a brief mention of flatworms by Qvarnstrom et al. (2013). To our knowledge, there have been no studies of rat lungworm in amphibians in Hawaii. Although Hawaii has no native amphibians, multiple species have been introduced, including the invasive Puerto Rican coqui frog (*Eleutherodactylus coqui*), Cuban greenhouse frog (*Eleutherodactylus planirostris*), and cane toad (*Rhinella marina*). In particular, coqui frogs have one of the highest densities of terrestrial amphibians worldwide, with densities in Hawaii reaching 91,000 frogs/ha (Beard et al. 2009). Deter-

mining the role that paratenic hosts may be playing in the transmission and life cycle maintenance of rat lungworm is an important component in the overall understanding of the epidemiology of rat lungworm disease. Here, we report observations of rat lungworm infections in coqui frogs in Hawaii, as well as preliminary findings in greenhouse frogs, cane toads, and the Chinese red-headed centipede (*Scolopendra subspinipes*).

We collected 24 coqui frogs on 21 June 2018 from a site in Hilo, Hawaii. The site is at an elevation of approximately 25 m and is in an area known to have high levels of rat lungworm infections in wild rats (Jarvi et al. 2017). Additionally, four greenhouse frogs, two cane toads, and three centipedes were collected opportunistically between February and June 2018 within 20 km of the coqui frog collection site. Tissue samples taken from frogs and toads comprised approximately 25–100 mg of each of five distinct regions: digestive tract, muscle, liver, heart, and brain. Typically, the brains and hearts were collected in their entirety, whereas digestive tract samples consisted of tissue from both stomach and lower intestine. Liver samples comprised tissue from each of the three lobes, and muscle samples included tissue from both thigh muscles. The centipede samples consisted of scrapings of internal organs from within the exoskeleton. Snout-vent length, or the length from tip of the snout to the posterior end of the backbone, was taken for each coqui frog to the nearest millimeter. For each individual sampled, separate instruments were used for the dissection of each organ after soaking in 10% bleach (Clorox 8.3% v/v sodium hypochlorite diluted 10:1) for a minimum of 10 min to remove any potential DNA contamination (Prince and Andrus 1992). Tissue samples were stored in 500 µL of DNA lysis buffer (0.1 M Tris HCl, 0.1 M ethylenediaminetetraacetic acid, 2% sodium dodecyl sulfate) with 0.2 g of 0.5-mm zirconia-silica beads (BioSpec Products, Bartlesville, Oklahoma, USA) and six 3.0-mm zirconia beads (OPS Diagnostics, Bridgewater, New Jersey, USA) at –80 C. Samples were homogenized (one to four cycles, 8 m/s, 40 s) in a FastPrep-24 5G bead-beater (MP Biomedicals, Santa Ana, California, USA), cooled on ice for 5 min, and centrifuged at 6,200 × G for 3 min. DNA was extracted from 50 µL of tissue homogenate using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) according to the manufacturer's animal tissue protocol. Tissue samples smaller than 25 mg were digested directly in 180 µL of DNA lysis buffer without homogenization. Real-time PCR followed Jarvi et al. (2012), with thermal cycling conditions of one cycle of 50 C for 2 min, 95 C for 10 min, followed by 40 cycles of 95 C for 15 s, 60 C for 1 min. Initial data showed amplification of some samples beginning after 35 cycles, so the number of cycles was increased to 50 cycles to allow for these samples to reach the plateau phase of amplification. Samples were considered positive for rat lungworm if two or three replicates within a run showed exponential amplification in both the ΔRn vs. cycle and Rn vs. cycle plot types that crossed a set threshold of 0.25 fluorescence units. One small brain sample was evaluated at a 0.015 threshold because of a lack of sample available to rerun at 50 cycles. Most replicates of a sample had a cycle threshold (C_T) SD<0.5 within one run; however, because consistent C_T replication is challenging with very low target DNA concentrations, a C_T SD of 0.5–1.0 within one run was accepted for sample replicates with cycle thresholds >35. Despite repeated attempts, replicates of two brain samples were unable to produce a C_T SD<1.0 within one run; however, multiple replicates were consistently positive in repeated runs (Table 1). Samples with low reproducibility were also deemed positive if one replicate per run showed amplification and was reproduced in multiple runs (Table 1). Samples with exponential amplification in only one replicate that was not reproduced in multiple runs were categorized as undetermined. Negative samples were determined by lack of exponential amplification in all replicates. All animal procedures followed the approved Institutional Animal Care and Use Committee protocol

TABLE 1. Presence (+) or absence (–) of rat lungworm (*Angiostrongylus cantonensis*) in different tissue types from invasive coqui frogs (*Eleutherodactylus coqui*) in Hilo, Hawaii, by real-time PCR.

			Tissue type analyzed[a]				
Frog no.	SVL (mm)	Status[b]	Brain	Heart	Liver	Muscle	Stomach-intestine
1	14	P	ND	–	+[c]	–	–
2	15	A	ND	ND	ND	UD	–
3	22	A	–	UD	–	–	–
4	26	P	ND	+	+	–	+
5	27	P	+	ND	+	+	+
6	28	P	–	–	–	–	+
7	28	P	–	+[c]	–	–	+
8	29	P	+	UD	–	–	+
9	30	P	–	+[c]	–	+	+
10	30	P	+	+	+[c]	–	+
11	31	A	–	–	–	–	–
12	31	P	+	+	+	+	+
13	32	P	+[c]	–	+	+	+
14	32	P	UD	–	+	+	–
15	33	P	+	+[c]	–	+	–
16	34	P	–	+	–	+	+
17	36	P	+	+	+	ND	+
18	37	P	ND	–	+	–	–
19	37	P	+	–	–	+	–
20	37	P	+[d]	+	+	+	+
21	38	P	+[d]	+	+	+	+
22	39	P	+	+	+	–	+
23[e]	39	P	–	+	+	+	+
24	42	P	–	–	+	+	+
% Positive[f]		87 (21/24)	58 (11/19)	60 (12/20)	61 (14/23)	55 (12/22)	67 (16/24)

[a] SVL = snout-vent length; ND = no data; UD = undetermined (samples with exponential amplification in only one replicate that was not reproduced in multiple runs).

[b] Presence (P) and absence (A) of *A. cantonensis* from one or more tissue types.

[c] Samples with low reproducibility, having one replicate per run reproduced in multiple runs.

[d] Samples with consistently positive replicates in repeated runs but a cycle threshold SD>1.0 across replicates within one run.

[e] Frog with semislug (*Parmarion martensi*) found in stomach that also tested positive for *A. cantonensis*.

[f] Total number of samples positive/total number of samples analyzed in each category.

(QA-2835, US Department of Agriculture, National Wildlife Research Center).

A total of 87% (21/24) of sampled coqui frogs were positive for rat lungworm in at least one tissue type per individual. Overall, parasite presence was detected in each of the five tissue types sampled (stomach-intestine, muscle, liver, heart, and brain), with 14 frogs positive for at least three tissue types (Table 1). Snout-vent lengths of coqui frogs ranged from 14 to 42 mm, with positive individuals found at both extremes. Of note, a whole semislug (*Parmarion martensi*) was found in the stomach of one of the frogs sampled, with both the frog and slug testing positive (Table 1). The invasive *P. martensi* can carry heavy parasite burdens and has been identified as a highly competent intermediate host of rat lungworm in Hawaii (Hollingsworth et al. 2007). Additionally, the four greenhouse frogs, two cane toads, and three centipedes were all positive for rat lungworm, with the latter previously identified as a paratenic host in China (Wang et al. 2018).

Although experimental infections may be necessary to confirm these species' role as paratenic hosts definitively, our findings suggest that they have the potential to be players in rat lungworm epidemiology in Hawaii. Although molecular analysis only confirms the presence of the parasite DNA and not life cycle stage or viability, positive detections from muscle, liver, heart, and brain (as opposed to stomach-intestine) indicate larval migration within the host's body, with some of these tissue types previously identified as the source of human infection from other frog species (Cuneo et al. 2006). Although the species discussed here are not known to be intentionally consumed by humans in Hawaii, the ingestion of infected hosts could still pose a threat to other animals, because rat lungworm can infect both domestic and wild animals such as dogs (*Canis lupus familiaris*), horses (*Equus caballus*), and birds (Spratt 2015).

If these species are indeed capable of acting as reservoirs for infective larvae, then there may also be spillover risk to rat definitive hosts, ultimately aiding in the completion of the parasite life cycle in the wild. Rats have been documented scavenging coqui frog and cane toad carcasses on Hawaii Island (Abernethy et al. 2016), where rat species have high rat lungworm infection levels (Jarvi et al. 2017). These rat species are also known to consume centipedes, cane toads, and *Eleutherodactylus* spp. elsewhere (Marples 1955; Fitzgerald 1990; Stewart and Woolbright 1996). Concern also exists regarding the potential spread of infected paratenic hosts to other locations, especially in areas where competent definitive and intermediate hosts are already present. Multiple reports exist of frogs, including coqui frogs, being spread from Hawaii to other locations such as Guam and the continental USA (Beard et al. 2009; Olson et al. 2012). Although our report of rat lungworm infections in frogs and centipedes implicates them as possible disease reservoirs, further investigations are warranted to better understand the role paratenic hosts may be playing in angiostrongyliasis transmission in Hawaii.

This study was funded by the US Department of Agriculture, University of Hawaii at Hilo, National Institutes of Health National Institute of General Medical Sciences, IDeA Networks of Biomedical Research Excellence (award P20GM103466), and the Hawaii Invasive Species Council. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of their respective institutions. We thank two anonymous reviewers and the editor for their comments that improved this manuscript.

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Submitted for publication 19 December 2018.
Accepted 4 April 2019.

Appendix B

Accounting of financial expenditures from HISC award. Other costs such as additional labor and molecular analyses were paid through in-kind expenditures by WS-NWRC and UH-Hilo School of Pharmacy.

Expense	**Amount**
Salaries	18,126.08
Benefits	6,759.12
Molecular analyses	8,000.00
Supplies & materials	124.79
S&B action in process	*$2,392.74*
Total Direct Expense	$35,402.73
Overhead*	$3,300.96
OH adjustement in process	*$239.31*
TOTAL	**$38,943.00**

Expenses for additional salaries, materials, vehicles, facilities, shipping, etc., were paid by WS-NWRC.