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The potential for rainwater catchment as a source of human infection by Angiostrongylus cantonensis in Hawaii

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Rat Lungworm Disease caused by *A. cantonensis* is a GLOBAL disease
Wang et al., 2008; 2012

*A. cantonensis* has been documented as a parasitic disease of humans in Hawaii and other Pacific islands since the early 1960’s (Wallace and Rosen 1969; Alicata & Jindrak 1970 and Alicata 1991).
Hawaii is the epicenter of rat lungworm disease in the United States.

- In Hawaii it is assumed that most human infections are the result of ingestion of an intermediate host on raw, unwashed or poorly washed produce.

- Angiostrongyliasis in humans can result in transient meningitis or a more serious disease involving the brain, spinal cord and nerve roots.

- 60 cases of human infection by *A. cantonensis* have been reported by the State Health Dept. since 2001. (Hochberg et al. 2007, Hawaii DOH). 90% of cases have originated on Hawaii Island.
**Life Cycle of Rat Lung Worm**

1. **rat eats snail/slug**
2. Ingests 3\(^{rd}\) stage worms
3. Worms penetrate intestine
4. Enter bloodstream
5. Mature in CNS
6. Reproduce in heart
7. Eggs move to lungs
8. Worms move to trachea, are swallowed and expelled in feces.
9. rat feces eaten by snails/slugs
10. Eggs hatch into 1\(^{st}\) stage worms, penetrate alveoli
11. Mature L1 to L3 in slugs
12. L3 larvae are infective to humans and others

**Adult A. cantonensis**
Important carriers of rat lungworm disease in Hawaii:


The **Cuban slug** *Veronicella cubensis* arrived ~1980. It is a serious agricultural pest.

The **giant African snail** *Achatina fulica* one of the best known invasive snail species. It was introduced into the Pacific Basin just prior to WWII.

Photo credits: Drs. David Robinson, Rob Hollingsworth
Paratenic hosts can be infective (transport hosts)

- Not obligatory to life cycle
- Larvae do not mature or reproduce in these hosts
- Can infect definitive, accidental or other paratenic hosts

Accidental hosts can become infected (dead-end hosts)

- Not obligatory to life cycle
- Dead end for the parasite - larvae do not reproduce in these hosts, but die
- Can often cause sickness or death
Of 37 non-native mollusk species screened in Hawaii, 16 tested positive for *A. cantonensis*. Of 7 native species, 2 tested positive. Infected mollusks were found at elevations up to 1203 m. (Kim 2013, Kim et al. 2014).

Figure 3.1. Presence (40 sites, numbers in parentheses) and absence (142 sites) of *Angiostrongylus cantonensis* at the study sites. Red – present; yellow – absent; some sites overlap and may not be visible. Green – low elevation; brown – high elevation.
Percentage of sites (of 182) testing positive for *A. cantonensis* (Kim 2013):

- Kauai 34%
- Hawaii 33%
- Maui Nui 18%
- Oahu 10%

*P. martensi* is only documented on Hawaii and Oahu

Catchment use on Hawaii vs Kauai?
Big Island Districts:

Number of households without municipal water

Percentage of households without municipal water

Slide kindly provided by Patricia MaComber
Some victims believe consumption of catchment water may have caused their infection.

No studies have been done in Hawaii to ascertain if this is possible.
Can catchment water be a source of infection?

•~ 30,000-60,000 people in the state of Hawaii rely on catchment water for household water; the majority are in the Puna, Ka’u, and Hamakua districts of the Island of Hawaii (Macomber 2010).

•Many reports of slugs/snails crawling up side and into catchment tanks.

•Cheng and Alicata 1964
  Possible for infective larvae shed by mollusks to contaminate water. After 22-26 hours, third-stage larvae observed in water. Torrential rainfall could lead to mollusk death by drowning. Theoretically possible to be infected by drinking water.

•Crook, Fulton, Supanwong 1971
  Thailand: well water contaminated with A. cantonensis from Achatina fulica observed around/ crawling/falling into wells. Drowned snails shed third stage larvae for up to 50 hours.
Richards and Merritt 1967

- L1 *A. cantonensis* larvae survived 3 weeks in freshwater and were infective to snails after 2 weeks in either freshwater or seawater.
- Rats became infected by drinking water containing L3 larvae, and L3 were active in freshwater up to a week.
- Infected *Biomphalaria glabrata* (freshwater snail) died when immersed in seawater, but yielded infective larvae up to 5 days.
- Marine hosts as intermediate hosts?
- Drinking water could be contaminated by “appreciable numbers” of third-stage larvae from digested or macerated infected snails...
10 day pilot study: where in a column of water would *A. cantonensis* larvae be found?

- We observe infective L3 larvae migrate down and into collection tubes attached to modified Baermann filters.
- Intake pipe for catchment tank is near bottom of tank.
Methods

• Rainwater directly off of a roof with no overhanging vegetation and no gutters, and transferred to the lab in a clean, half-gallon glass Mason jar.
• 10 *P.martensi* (5=W, 5=M) in Falcon tubes with 50 mLs of rainwater (5 macerated, 5 whole).
• 5 mL samples taken from bottom, middle, and top of water column and put into petri dish. (10 slugs x 3 locations=30 samples).
• Rainwater added to Falcon tube to maintain 50 mL level.
• Rainwater samples with no slugs added were tested in parallel.
• Samples examined with dissecting microscope.
• A molecular test was used to quantify RLW DNA from mollusks.
Number of larvae pulled from 50 ml Falcon tubes up to day 5
Most larvae originated from whole (vs. macerated) slugs.

Moving vs. non-moving larvae over a 7 day period of time. Non-moving worms were either not yet emerged (early) or dead (late).

<table>
<thead>
<tr>
<th>Day</th>
<th>Moving</th>
<th>Non-moving</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0</td>
<td>400+</td>
<td>400+</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>296</td>
<td>371</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>&gt;600</td>
<td>675</td>
</tr>
<tr>
<td>6</td>
<td>151</td>
<td>286</td>
<td>437</td>
</tr>
<tr>
<td>7</td>
<td>186</td>
<td>406</td>
<td>592</td>
</tr>
<tr>
<td>8</td>
<td>242</td>
<td>379</td>
<td>621</td>
</tr>
<tr>
<td>9</td>
<td>322</td>
<td>626</td>
<td>645</td>
</tr>
<tr>
<td>10</td>
<td>241</td>
<td>937</td>
<td>1178</td>
</tr>
</tbody>
</table>
Number of *A. cantonensis* larvae collected 5 mL samples and their location in the water column (day 5 estimates are not included). 92% of larvae were found in bottom samples.
Conclusions

• Greater numbers of larvae were released from whole *P. martensi* slugs as opposed to macerated slugs; could be due to variance in levels of infection (don’t have to be macerated).
• Greatest number of larvae were from 5 mL samples taken from the bottom of 50 mL falcon tubes.
• Viable L3 stage larvae of *A. cantonensis* can be released from slug tissue as swimming L3 or encased in their molt sheaths and in coiled form.
• Larvae can emerge from coiled form and survive in rainwater for at least 10 days.
• Confirmation of infection by *A. cantonensis* in mollusks was made by quantitative PCR (qPCR).
2\textsuperscript{nd} water study to verify results of pilot study

Increase diversity of potential host species (13 specimens total):

- *Achatina fulica* (Giant African Snail)
- *Laevicaulus alte* (Leatherback slug)
- *Veronicella cubensis* (Cuban slug)
- *Parmarion martensi* (semislug)
- *Platydemus manokwari* (flatworm)

Increase study time to 20 days.
Methods

• Rainwater was collected as before, and inspected on a daily basis by microscopy and a sample was tested by qPCR to confirm the absence of RLW larvae
• 12 mollusks, one planarian were placed in Falcon tubes with 50 mLs of rainwater.
• Rainwater samples with no slugs added were tested in parallel.
• One half of each species was left whole, the other macerated.
• qPCR was used to determine infection level in mollusks.
• Five mL samples were drawn ~ 14 hours after cessation of mollusk movement from the bottom, middle, and top of each of the 50 mL Falcon tubes.
• Samples examined with dissecting microscope. Procedure repeated daily for 20 days.
Location of 5 mL sample taken in 50mL falcon tube water column, and numbers of larvae in those samples.
Visual results for nematode larvae and qPCR results from tail snips for all specimens used in trial. Overall, six of the 12 mollusks yielded variable amounts of nematodes.

<table>
<thead>
<tr>
<th>Species</th>
<th>tail snip qPCR</th>
<th>petri dish visual</th>
<th>Condition</th>
<th>Location collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>juv. A. fulica</td>
<td>positive</td>
<td>negative</td>
<td>whole</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>juv. A. fulica</td>
<td>negative</td>
<td>negative</td>
<td>macerated</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>A. fulica</td>
<td>negative</td>
<td>negative</td>
<td>macerated</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>A. fulica</td>
<td>negative</td>
<td>low positive</td>
<td>whole</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>L. alte</td>
<td>positive</td>
<td>high positive</td>
<td>macerated</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>L. alte</td>
<td>positive</td>
<td>low positive</td>
<td>whole</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>V. cubensis</td>
<td>negative</td>
<td>low positive</td>
<td>macerated</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>V. cubensis</td>
<td>negative</td>
<td>low positive</td>
<td>whole</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>P. manokwari</td>
<td>negative</td>
<td>negative</td>
<td>whole</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>P. martensi</td>
<td>negative</td>
<td>negative</td>
<td>macerated</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>P. martensi</td>
<td>negative</td>
<td>high positive</td>
<td>whole</td>
<td>Koa’e</td>
</tr>
<tr>
<td>P. martensi</td>
<td>negative</td>
<td>negative</td>
<td>macerated</td>
<td>Wa’a Wa’a</td>
</tr>
<tr>
<td>P. martensi</td>
<td>positive</td>
<td>positive</td>
<td>whole</td>
<td>Wa’a Wa’a</td>
</tr>
</tbody>
</table>

*L. alte* may require maceration to release large numbers of larvae, while *P. martensi* does not.
Larvae count from day 1 samples taken from *L. alte* (macerated) collected in Waa Waa area and *P. martensi* (whole) collected in Koae area.

20 day count from day 1 samples

Viable nematodes were observed over a 20 day period

What are they? (movie)
Variation in larval size or worms found in 5 mL samples taken from 50 mL falcon tubes containing a drowned slug (10x).
Opening a “can of worms.”

- One *L. alte* and two *P. martensi* showed high infection by nematodes.
- Greatest number of larvae were from samples taken from the bottom of 50 mL falcon tubes.
- Larvae were shed from mollusks up to Day 11.
- 5 mL samples pulled from 50 ml tubes on Day 1 contained only several nematodes initially, but
  - For *P. martensi*, peak larvae yield occurred D4-D5 then decreased.
  - For *L. alte*, peak larvae yield occurred Day 7 and was variable.
  - *L. alte* may require maceration to release large numbers of larvae, while *P. martensi* does not.
- Worms were various sizes and shapes.
Measurement of *A. cantonensis*

Ash, 1970 Summary of measurements of 35 L3 stage larvae (microns)

**Length**: 474 (425-524)

**Maximum width**: 26 (23-34)

Mackarras and Sandars 1955 (microns)

<table>
<thead>
<tr>
<th></th>
<th>Length</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>300</td>
<td>15</td>
</tr>
<tr>
<td>L2</td>
<td>450</td>
<td>30</td>
</tr>
<tr>
<td>L3</td>
<td>450</td>
<td>&lt;30</td>
</tr>
<tr>
<td>L4</td>
<td>1000</td>
<td>40</td>
</tr>
<tr>
<td>L5</td>
<td>2000</td>
<td>60</td>
</tr>
</tbody>
</table>

A 20 micron filter is recommended by the CDC for catchment systems (Macomber 2010). Is a 20 micron filter sufficient for a catchment system?
Methods

This study was the initial attempt to determine if larvae could pass through a 20 micron metal sieve.

- Two *P. martensi* and two *L. alte* (Trial I) and three *L. alte* and one *P. martensi* (Trial II) were tested by qPCR and found to be positive for *A. cantonensis*.
- Specimens were prepared similarly to those in the 10 and 20 day studies, except pre-rinsed with tapwater.
- Mollusks were placed in Falcon tubes with 50 mLs of rainwater.
- Three samples (5 mL) were drawn from the bottom of the 50mL falcon tube each day and put into petri dishes with 10mL of rainwater.
- Samples examined with dissecting microscope.
- Sieve was placed in a clean petri dish and immersed with rainwater.
- Larvae were placed on top of the sieve.
- Water from under the sieve was examined by microscopy and qPCR.
- Study was replicated with same results.
Results

Trial I Trial was terminated at Day 53 and Trial II at Day 56 (replicate) after first observation of worms emerging in water sample taken from 50 ml Falcon tubes with slugs.

DNA was extracted from samples collected from under the sieve, and tested by qPCR for the presence of A. cantonensis DNA. Both were positive (Trial I in replicate, Trial II quadruplicate). (movie)
Conclusions

Drowned mollusks can shed viable *A. cantonensis* larvae in rainwater.

Most larvae are found in the bottom of a water column.

Viable L3 stage larvae of *A. cantonensis* can be released from slug tissue as swimming larvae or in coiled form.

Larvae were shed from mollusks up to Day 11.

Days of peak larvae yields differed between species.

Viable *A. cantonensis* larvae were able to pass through a 20 micron sieve and live for at least 56 days.

Additional studies need to be completed.
What can we do to help reduce RLWD?

• **Learn more about RLWD on Hawai`i Island.**
  
  – **Objective 1.** Conduct studies to find out where the RLW ‘hotspots’ are.
    • Develop test to estimate the number of larvae in slugs and snails and bring information to public
  – **Objective 2.** Determine how effective various solutions are at killing (immobilizing) A. cantonensis larvae isolated from slugs.
    • Many people are using hydrogen peroxide or vinegar to wash vegetables- do they work?
  – **Objective 3.** Evaluate the possibility of RLW transmission in water.
  – **Objective 4.** Pilot study: Determine if RLW DNA can be detected in blood and optimize immunodiagnostic methods for detection of antibodies and/or antigens.
    • We need diagnostic tests to determine infection status available on Hawai`i island
  – **Objective 5.** Determine efficacy of a vaccine against A. costaricensis to A. cantonensis.
    • We might break the transmission cycle by vaccinating rats
  – **Objective 6.** Conduct a pilot study to determine the prevalence of human rat lungworm (RLW) infection in East Hawaii Island.

• **Educate friends, family and the public**
  
  – **Objective 7.** Integrate RLW education into public school system, increase public awareness
Acknowledgements

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References

Mahalo

Questions?