Detection of RLW in intermediate, definitive and paratenic hosts (molecular and other methods)

Yvonne Qvarnstrom

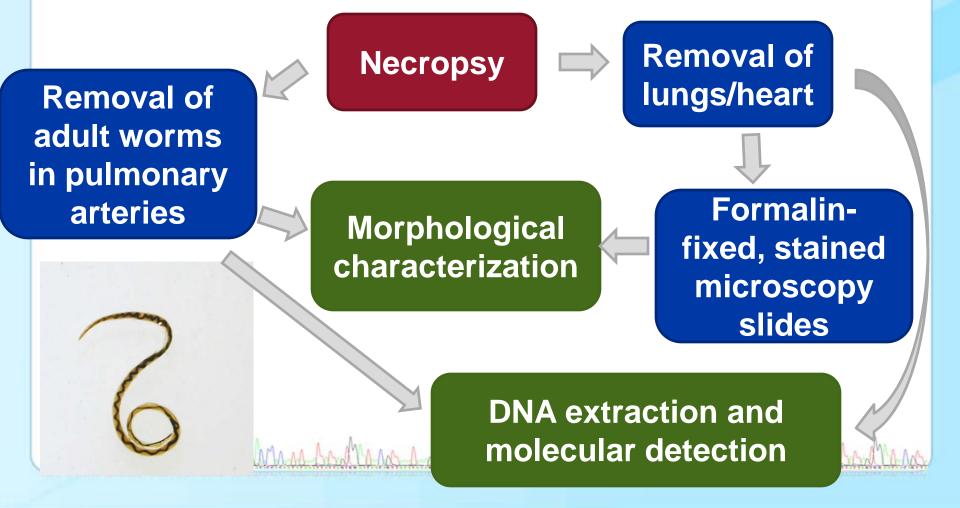
Senior service fellow, CDC

Rat lung worm disease scientific workshop August 18, 2011



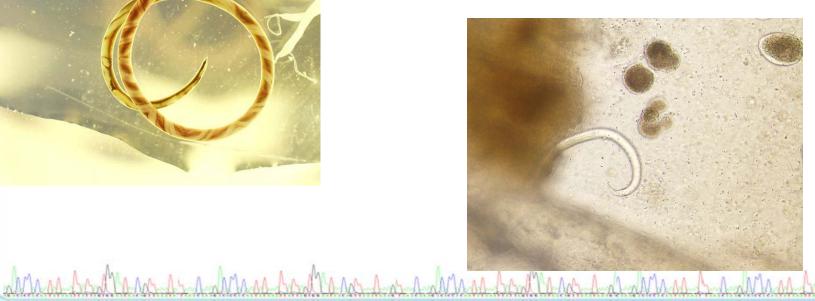
Methods for A. cantonensis detection

Adult worms in definitive hosts (rats)









Methods for A. cantonensis detection

L3 larvae in intermediate and paratenic hosts:

DNA extraction and molecular detection

direct visualization of nodules in lung tissue

Pepsin digestion to release L3



Adult worms in pulmonary arteries

rats with L3

Immature worms in brain



Morphological characterization





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Methods for A. cantonensis detection

L1 larvae in feces from definitive hosts (rats):

Infecting mollusks with L1



DNA extraction and molecular detection



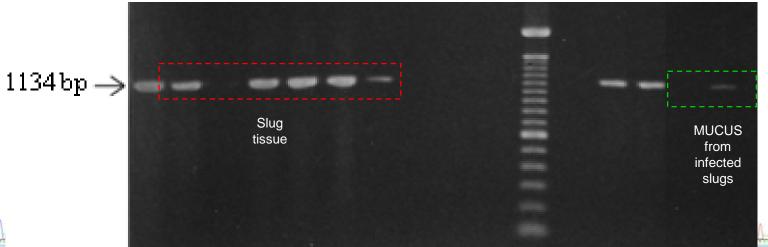
Detection of L3 in mollusks

- DNA extraction from worms:
 - phenol/chloroform
 - NaOH direct lysis
 - SDS/proteinase K digestion
 - Commercial extraction kits (Wizard, QIAamp...)
- DNA extraction from mollusks, stool etc:

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- Problems with amplification inhibition
- FastDNA kit, DNeasy tissue kit

- PCR and agarose gel detection
 - SSU rRNA
 - ITS2
 - CO1 (mitochondrial cytochrome c oxidase subunit I)
 - Not species-specific

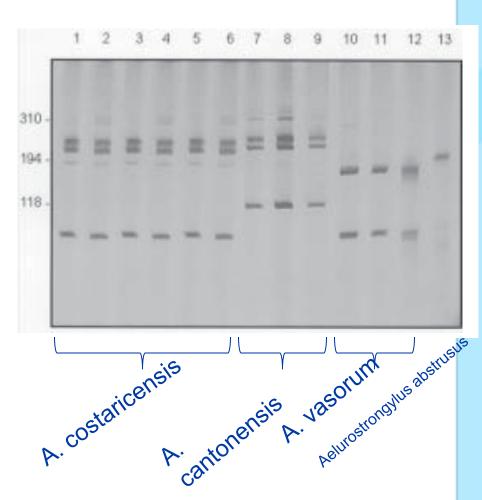




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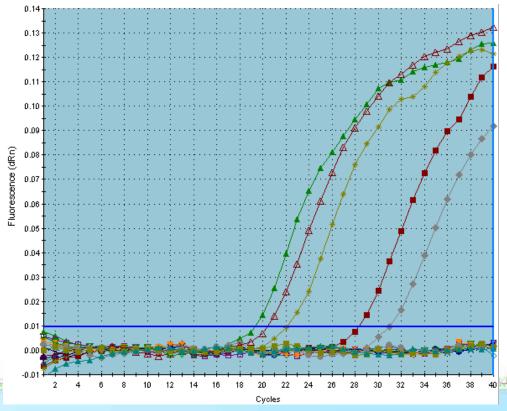
- PCR + sequencing
 - phylogenetic analysis

- PCR + RFLP
 - ITS2 PCR + Clal digestion



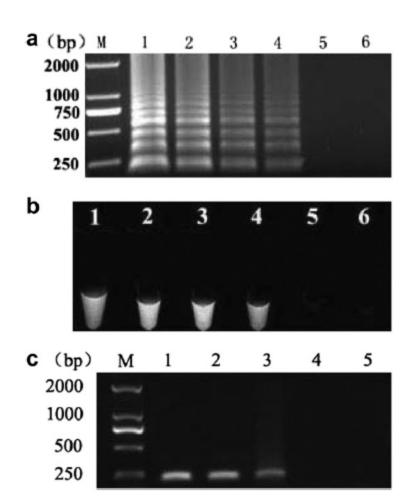
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- Real-time PCR
 - Species-specific primers and TaqMan probe
 - -ITS1
 - -ITS2



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- LAMP
 - Isothermal amplification
 - Bst DNA polymerase
 - Relies on six specific oligonucleotides
 - -ITS1



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Hawaii rats

- 2009: 28 rats total
 - -3 areas ●
 - Visual inspection:48% positives
 - Real-time PCR:100% positive
- 2011: 9 rats total
 - -3 areas ●
 - Visual inspection:89% positive



-1-30 adult worms/rat

Hawaii mollusks, 2005

species	Total number	Number of positives
Parmarion martensi	112	83 (74%)
Veronicella cubensis	50	22 (44%)
Laevicaulis alte	5	4 (80%)
Achatina fulica	6	5 (83%)
Other/unknown molluscs	16	8 (50%)
slime from infected slugs	13	1 (8%)
flatworms	2	2 (100%)

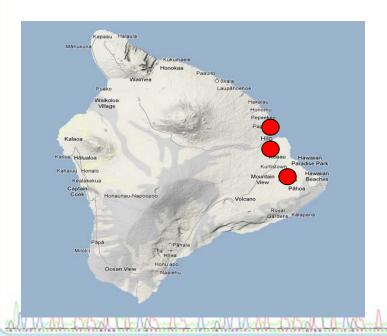






Hawaii mollusks, 2011





- Schofield Marine Base, Oahu:
 - Achatina fulica: 5 +/9 total
- Residents of patients, Big Island:
 - Cuban slug: 5+/22 total
 - Parmarion: 1 +/1 total
 - "worms": no +/7 total

Haiti 2002:

- 19 Rattus rattus
 - 4 positive
- 4 Rattus norvegicus
 - 3 positive



- 192 Rattus rattus trapped
 - 45 positive
 - All six surveyed geographical areas affected

Canary Island, 2010:

- 70 mollusks, various species
 - No A. cantonensis larvae
- 67 Rattus rattus
 - 10 positive, PCR confirmed
 - Only from one geographic location



Paratenic hosts

- Yellow tree monitor lizards, Thailand 1994:
 - 21 positive for L3 out of 22 total
 - 5 provinces covered
- Japan 2000:
 - Flatworms 14% positive (227/1613)
 - Amphibians 6% positive (1/18)
- National survey, China 2006/2007:
 - Paratenic hosts from markets and restaurants
 - Frogs, shrimps, crabs, toads, fish
 - None infected

