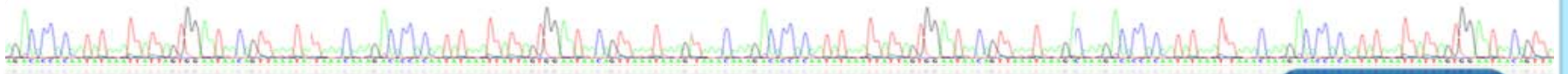


Detection of RLW in intermediate, definitive and paratenic hosts (molecular and other methods)

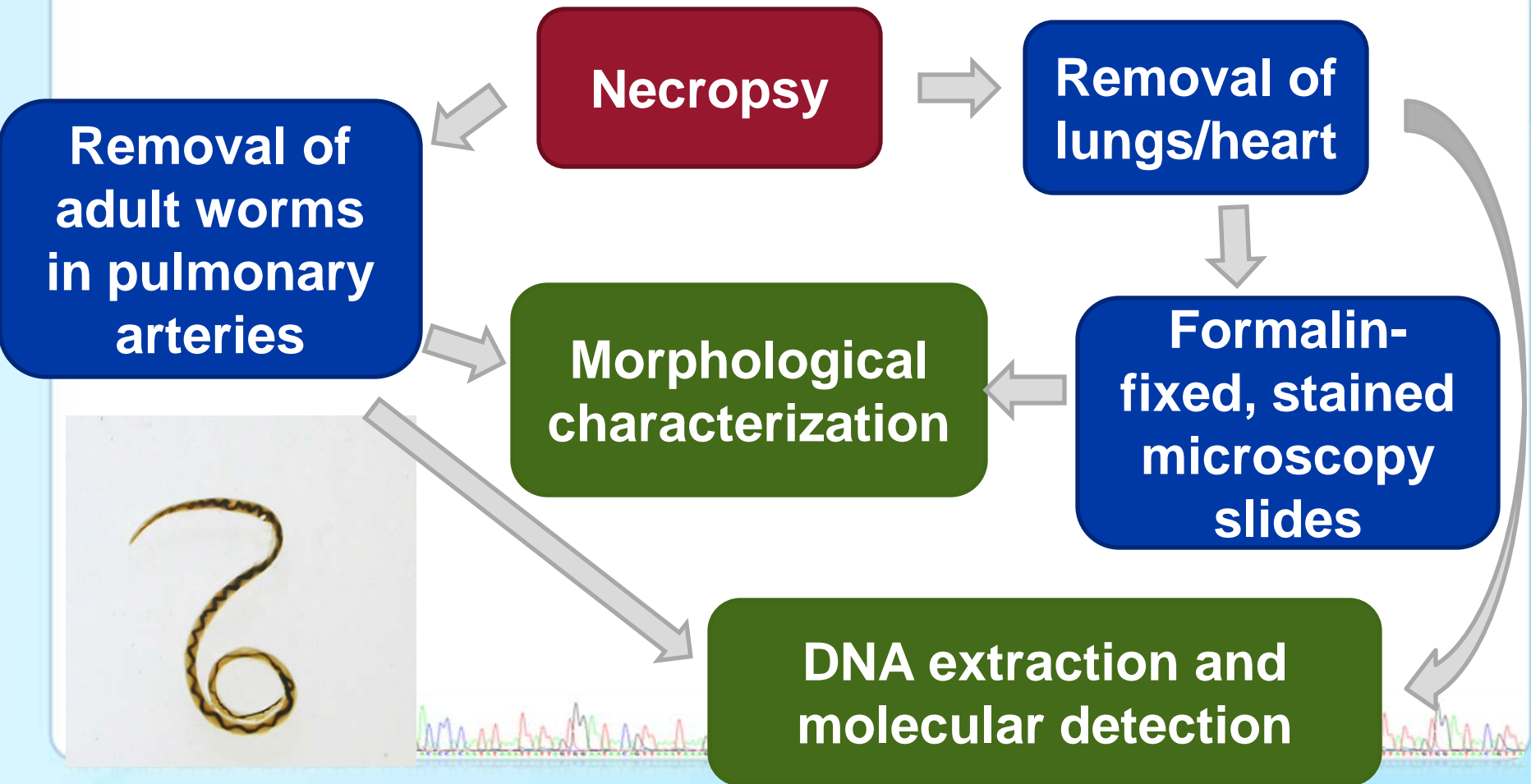
Yvonne Qvarnstrom
Senior service fellow, CDC

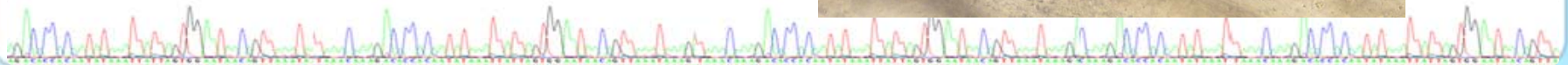
Rat lung worm disease scientific workshop
August 18, 2011



Methods for *A. cantonensis* detection

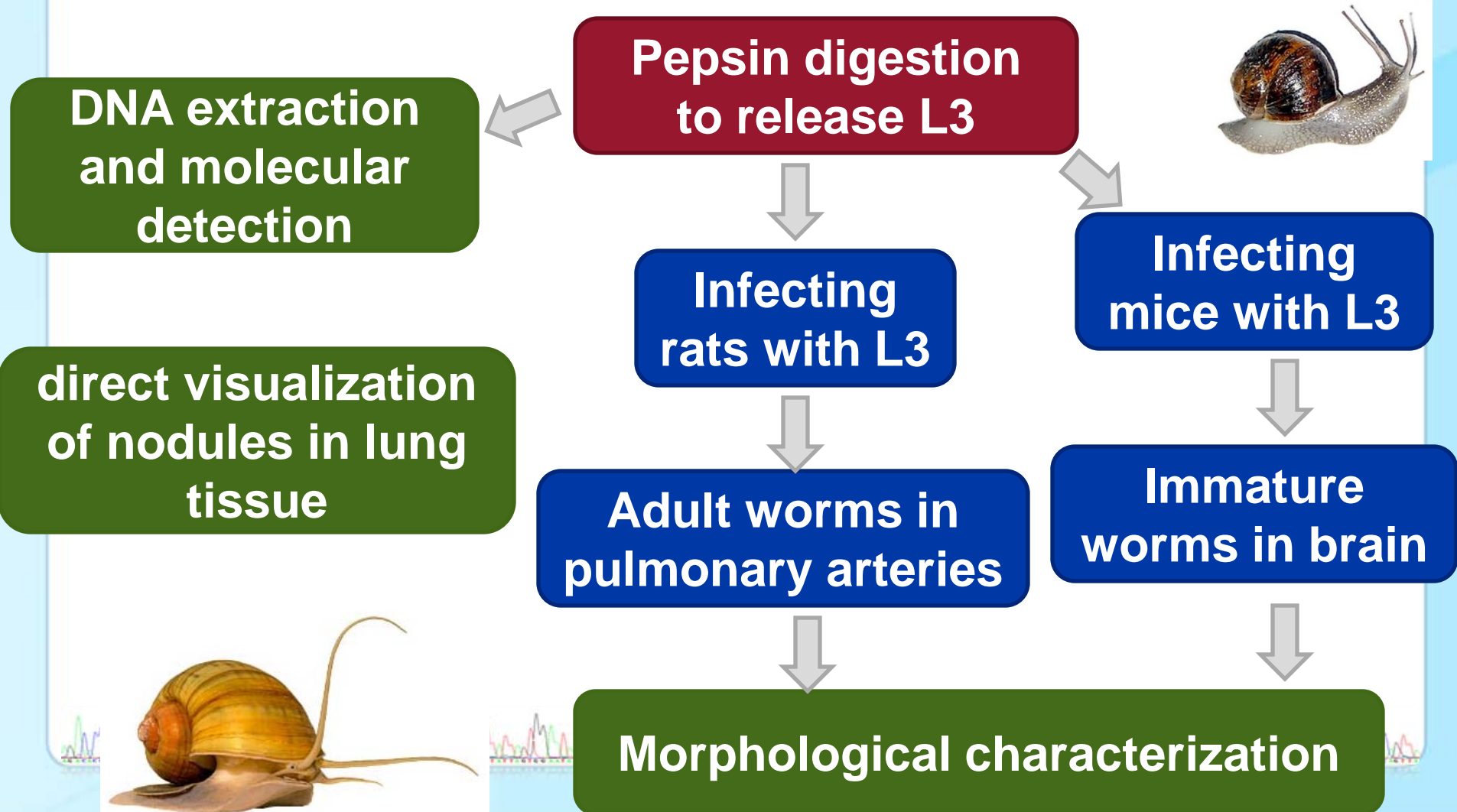
- Adult worms in definitive hosts (rats)

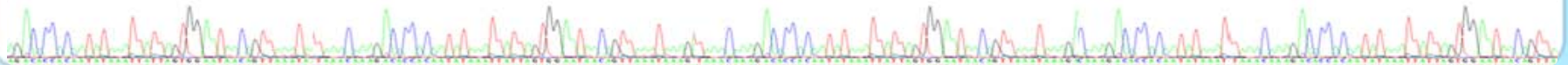
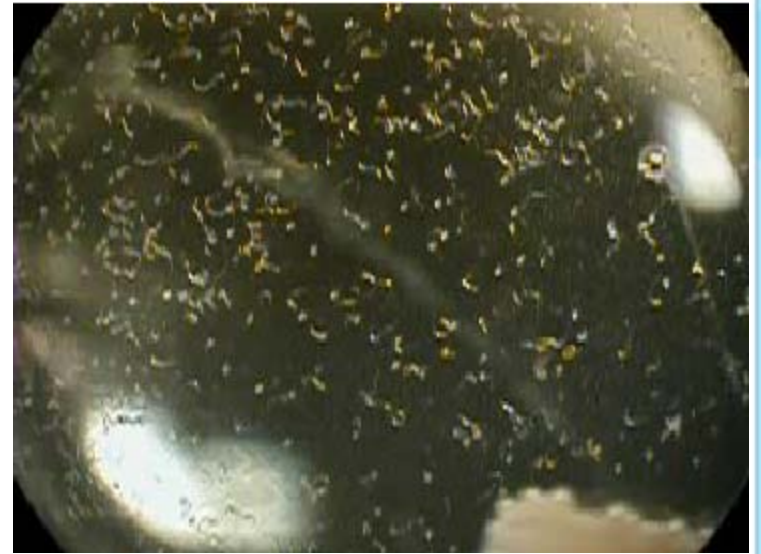


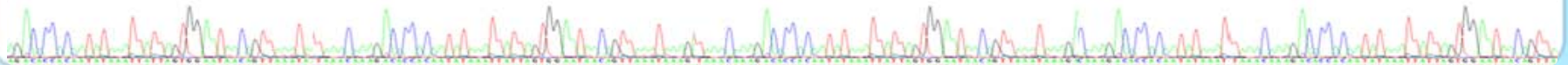
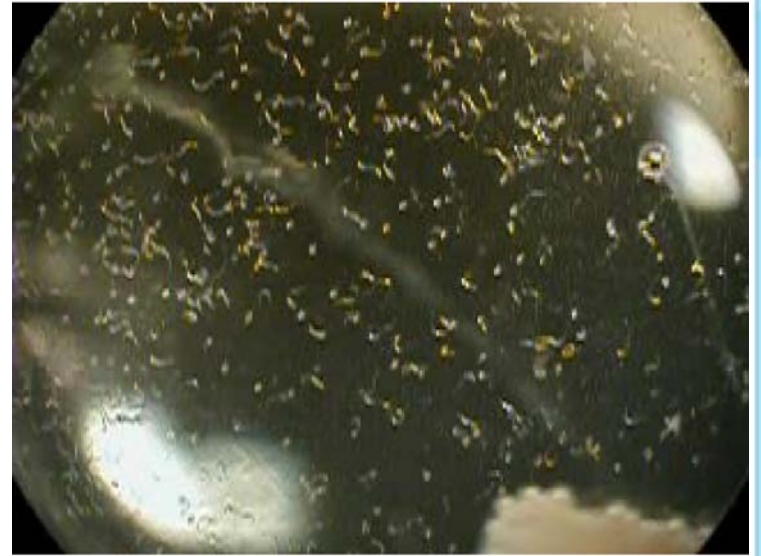


Methods for *A. cantonensis* detection

- L3 larvae in intermediate and paratenic hosts:

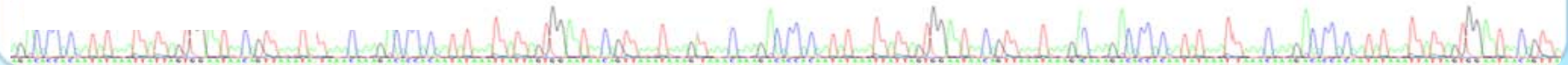
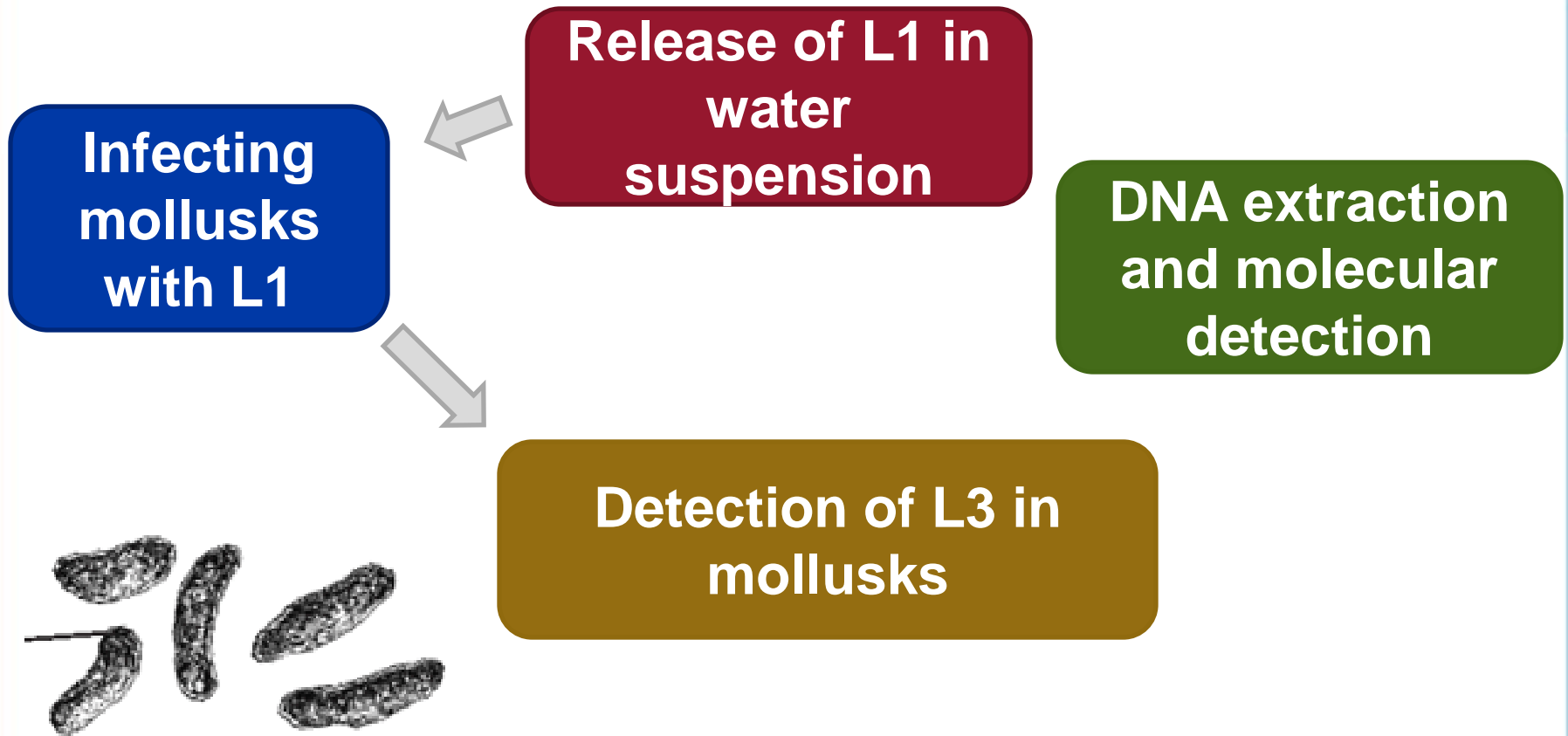






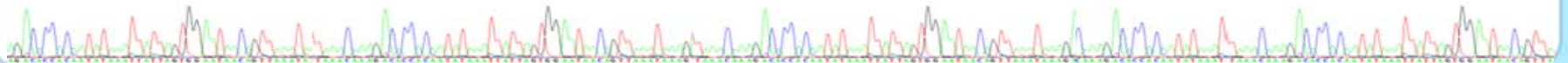
Methods for *A. cantonensis* detection

- L1 larvae in feces from definitive hosts (rats):



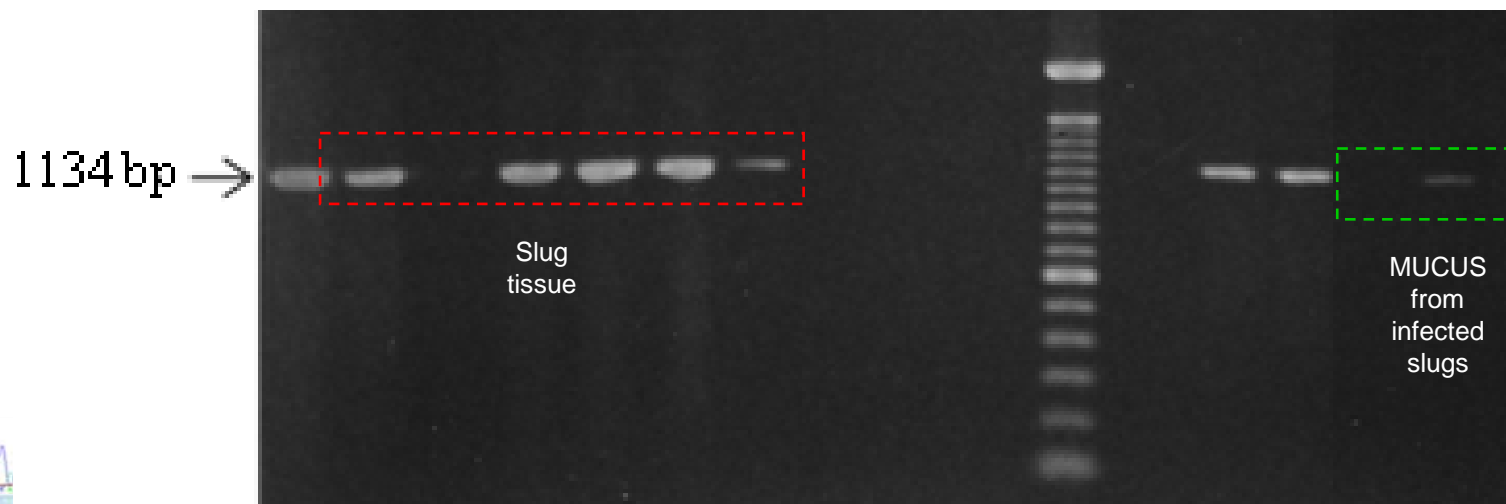
Molecular detection

- DNA extraction from worms:
 - phenol/chloroform
 - NaOH direct lysis
 - SDS/proteinase K digestion
 - Commercial extraction kits (Wizard, QIAamp...)
- DNA extraction from mollusks, stool etc:
 - Problems with amplification inhibition
 - FastDNA kit, DNeasy tissue kit



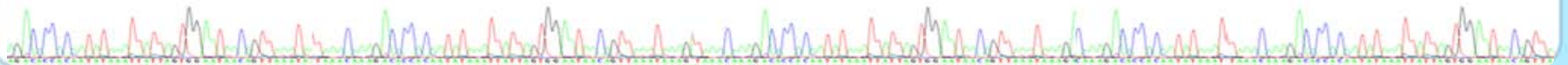
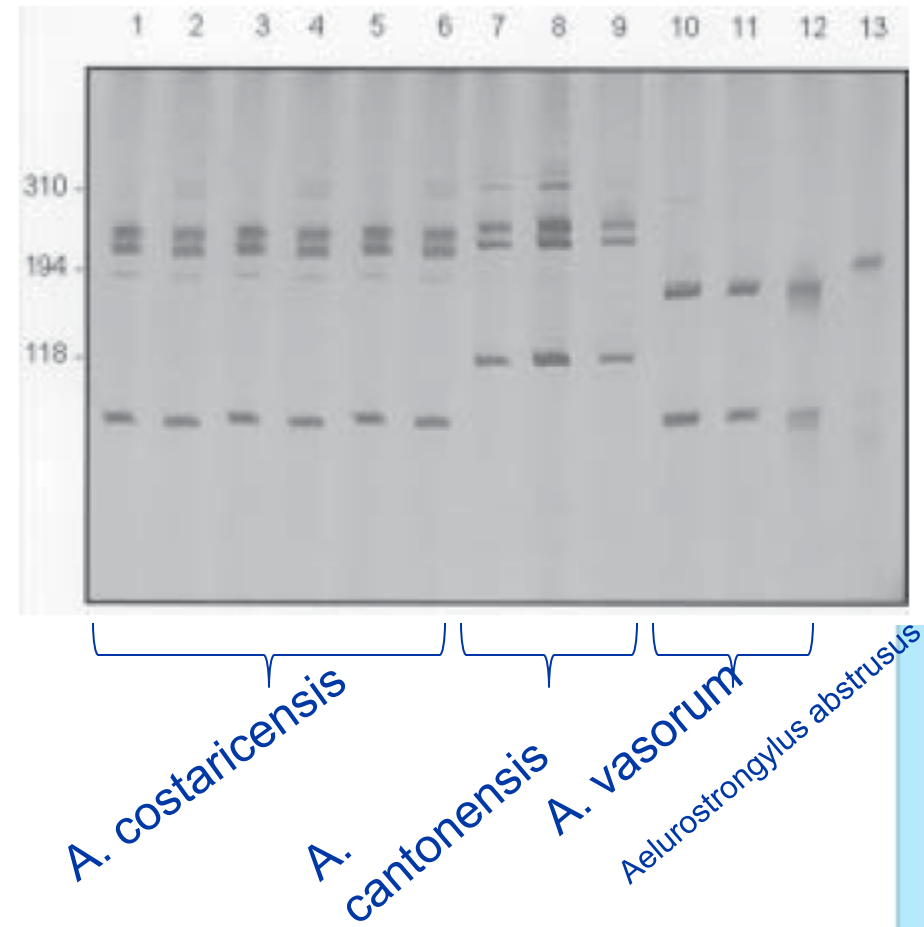
Molecular detection

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



Molecular detection

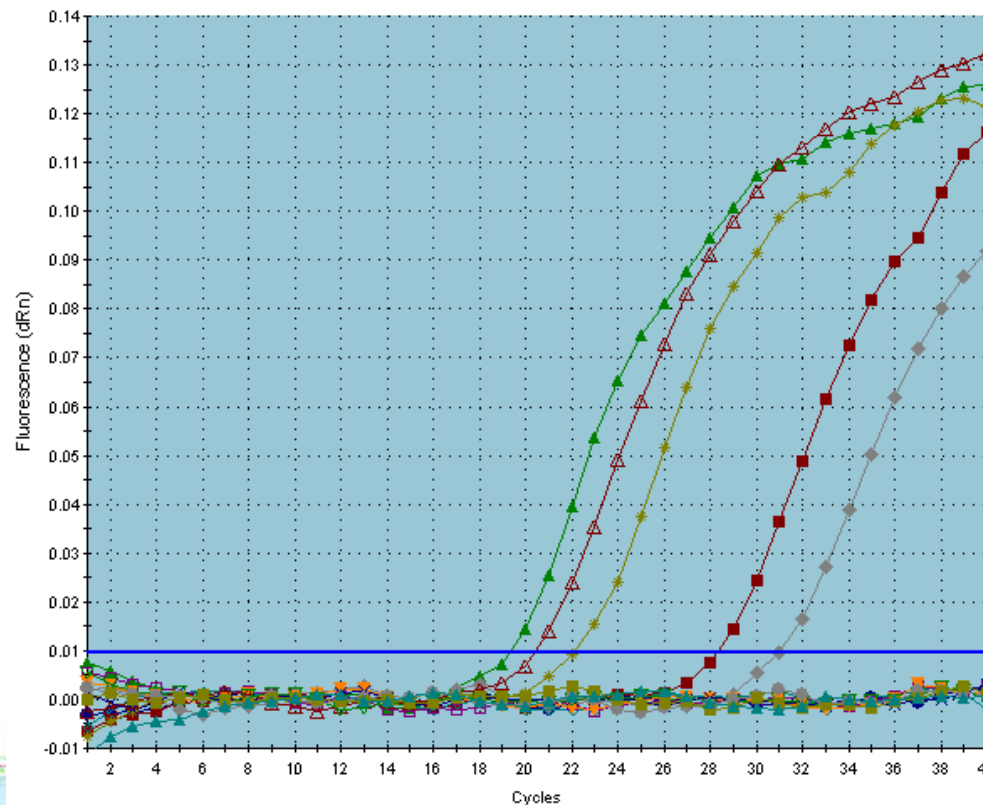
- PCR + sequencing
 - phylogenetic analysis
- PCR + RFLP
 - ITS2 PCR + *Cl*I digestion



Molecular detection

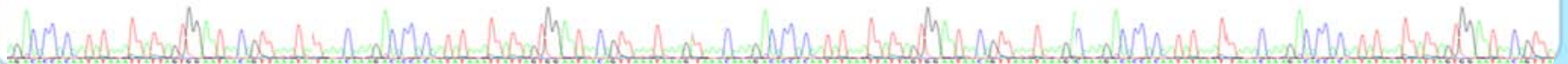
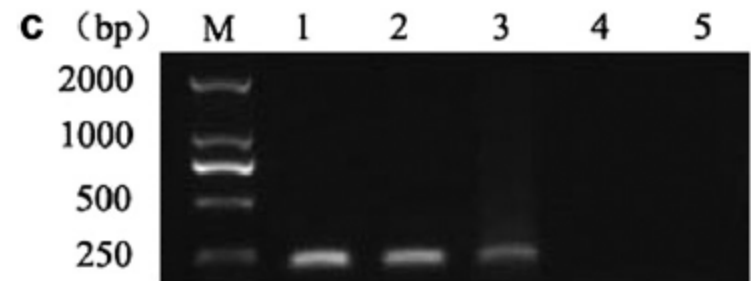
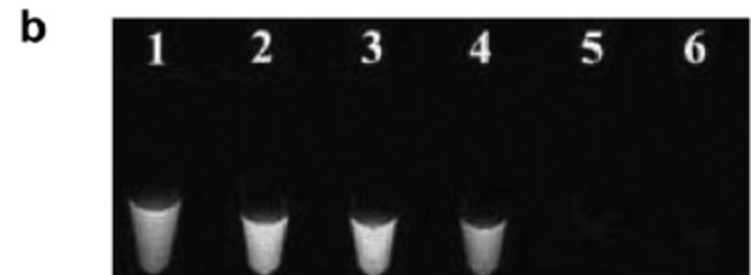
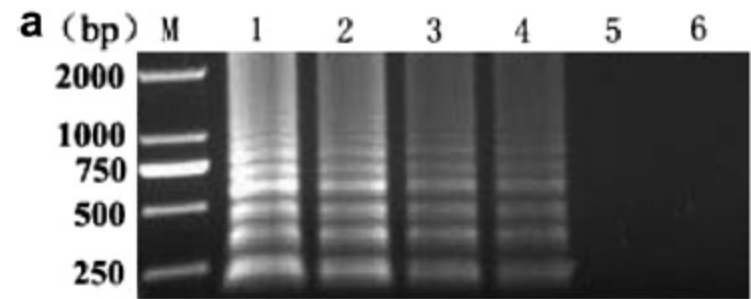
- Real-time PCR

- Species-specific primers and TaqMan probe
- ITS1
- ITS2



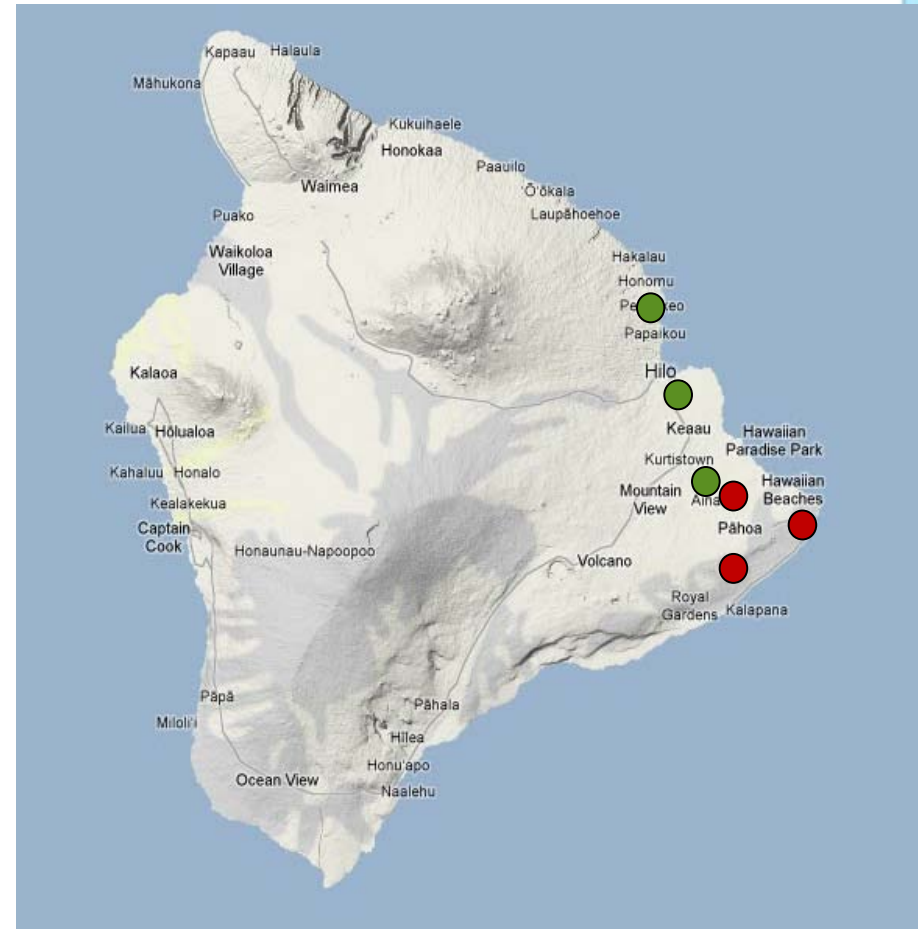
Molecular detection

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



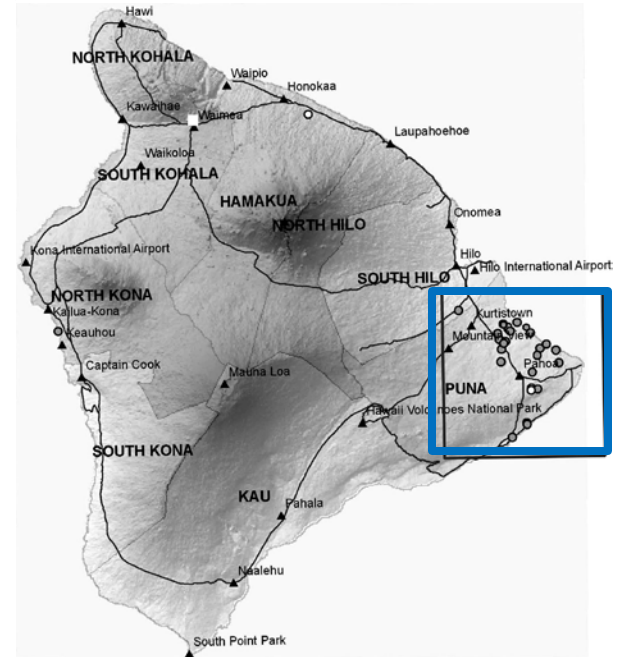
Hawaii rats

- 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
<i>Parmarion martensi</i>	112	83 (74%)
<i>Veronicella cubensis</i>	50	22 (44%)
<i>Laevicaulis alte</i>	5	4 (80%)
<i>Achatina fulica</i>	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:

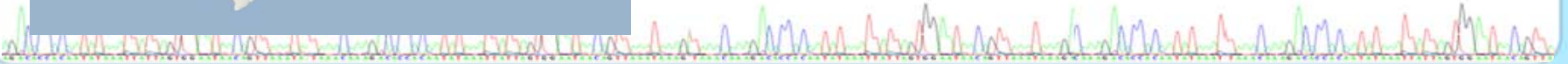
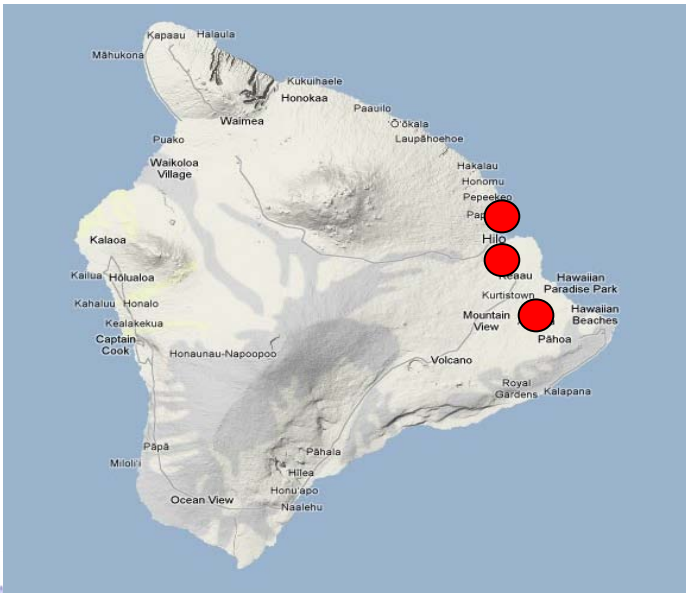
- *Achatina fulica*: 5 +/-9 total

- 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



Grenada, 2005-2006:

- 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



