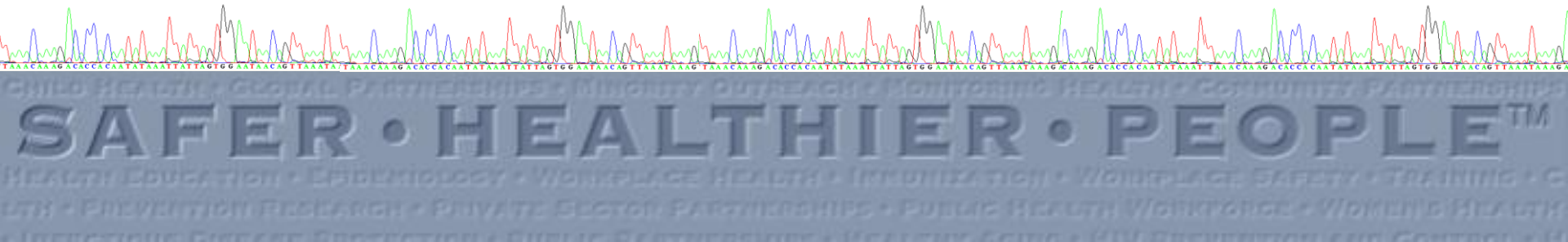


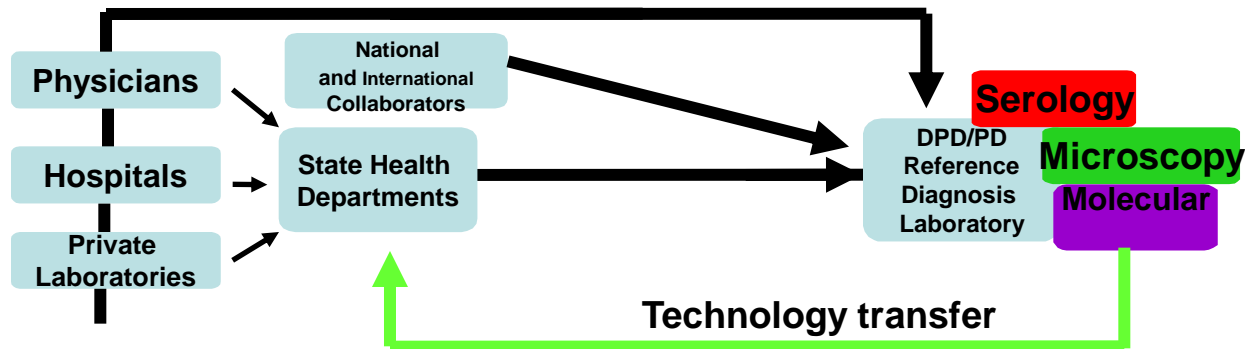
Detection of RLW and trends in geographic distribution

**RAT LUNG WORM DISEASE SCIENTIFIC WORKSHOP - HONOLULU,
HAWAII
AUGUST 16 - 18, 2011**

**Alex J. da Silva
DPDx/Molecular Diagnostics
Division of Parasitic Diseases and Malaria
Centers for Disease Control and Prevention
Atlanta, USA**



Reference Diagnostic Laboratories CDC/Parasitic diseases



Molecular diagnostics/DPDX mission

Provide high profile reference diagnostic service

Address significant gaps in diagnostic parasitology

Enhance workforce quality in diagnostic parasitology

DNA-based detection

DNA sequencing analysis of PCR-amplified fragments

Several distinct pathogens – based on matches with DNA sequences databases

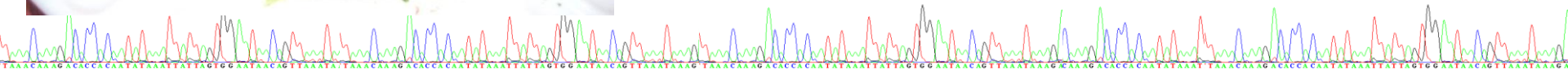


Sample

Blood
Tissue
CSF
Stool
Urine
Sputum
Liver aspirate
Water
Food
Slug/Snail
Animal material

PCR/real-time PCR

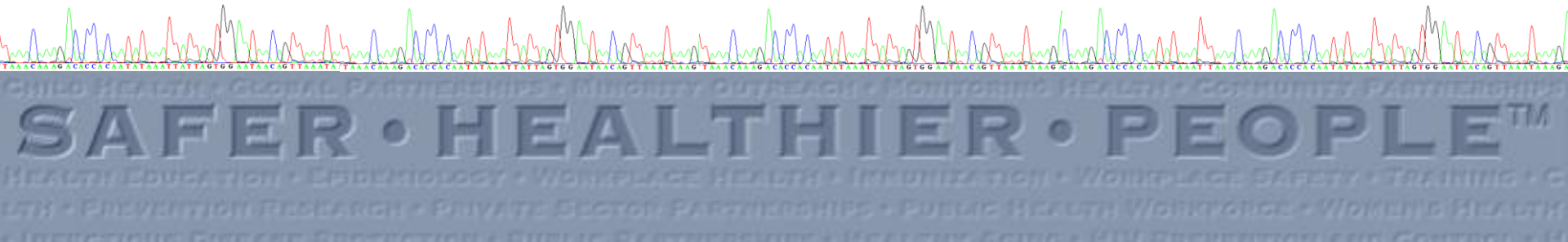
Amebiasis
Angiostrongyliasis
Babesiosis
Chagas disease
Cryptosporidiosis
Cyclosporiasis
Free-living amebic infections
Giardiasis
Leishmaniasis
Malaria
Microsporidiosis
Toxoplasmosis



Detection of RLW and trends in geographic distribution

A total of 21 species of the genus *Angiostrongylus* has been identified. Among them only two are of public health importance

1. *Angiostrongylus vasorum*
2. *Angiostrongylus raillieti*
3. *Angiostrongylus tateronae*
4. *Angiostrongylus ondatrae*
5. *Angiostrongylus cantonensis*
6. *Angiostrongylus ten*
7. *Angiostrongylus gubernaculatus*
8. *Angiostrongylus blarini*
9. *Angiostrongylus soricis*
10. *Angiostrongylus chabaudi*
11. *Angiostrongylus sciuri*
11. *Angiostrongylus sciuri*
12. *Angiostrongylus michiganensis*
13. *Angiostrongylus sandarsae*
14. *Angiostrongylus mackerrasae*
15. *Angiostrongylus malaysiensis*
16. *Angiostrongylus costaricensis*
17. *Angiostrongylus siamensis*
18. *Angiostrongylus schmidtii*
19. *Angiostrongylus minutus*
20. *Angiostrongylus dujardini*
21. *Angiostrongylus morerae*



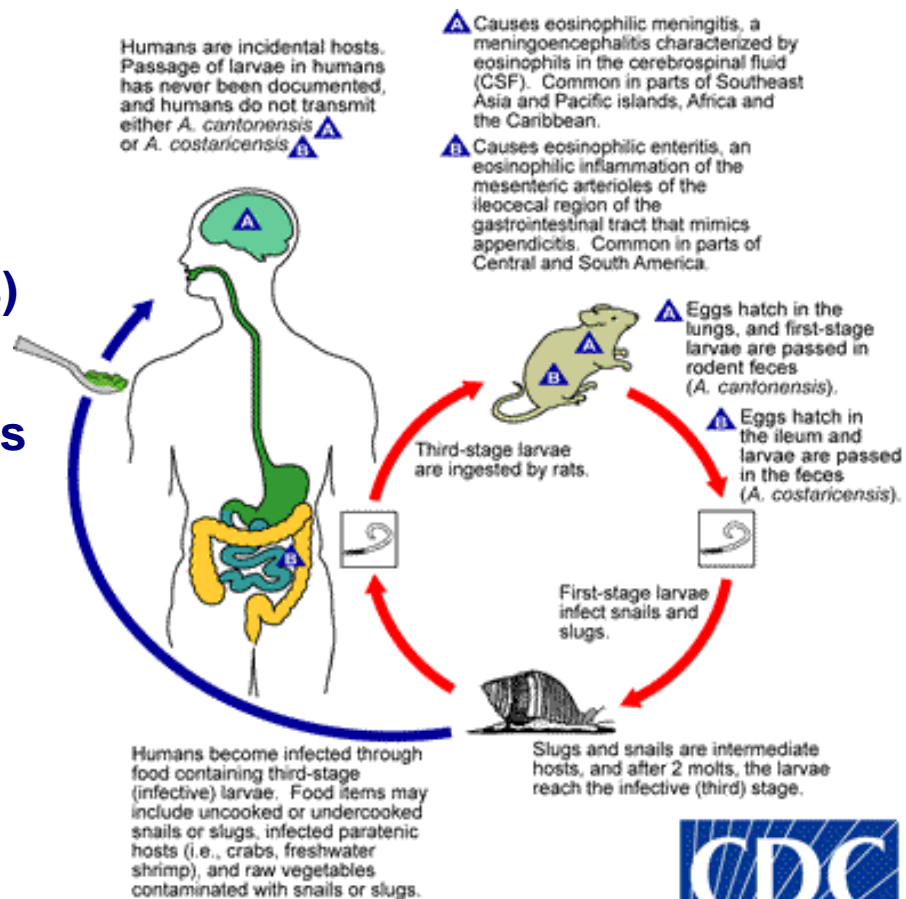
Angiostrongylus cantonensis

First described in 1933 in pulmonary arteries and hearts of rats

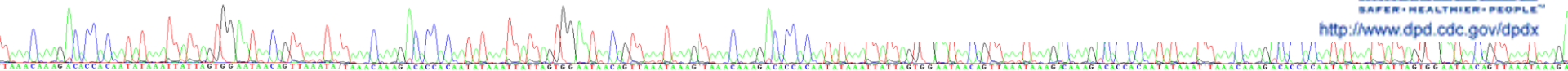
Thousands of cases have been reported worldwide (sporadic cases and outbreaks)

By 2008 over 2,800 cases had been documented in approximately 30 countries

The disease is emerging due to several factors; e.g., long distance transportation of intermediate and definitive hosts.



<http://www.dpd.cdc.gov/dpdx>



Angiostrongylus cantonensis

Laboratory Diagnosis:

Detection of antibodies in serum or CSF

Microscopy (limited)

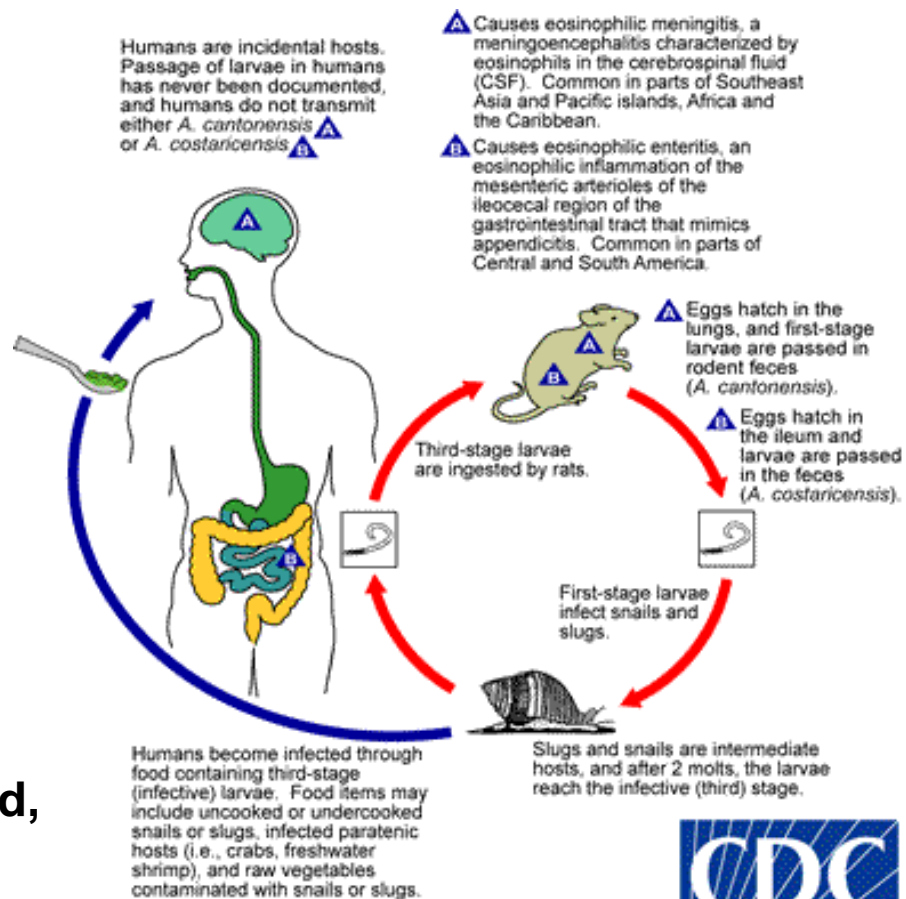
Molecular diagnostics

Environmental detection:

Morphology

Molecular

Samples for studies have to be collected, preserved and processed adequately



Angiostrongylus cantonensis

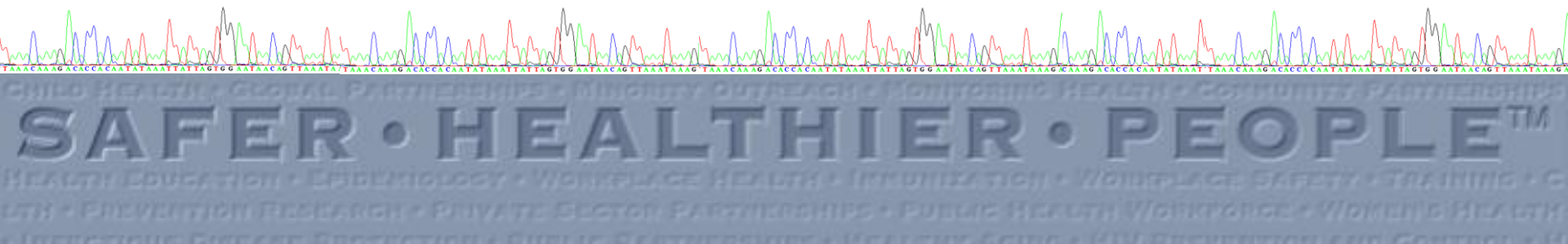
Table 1 Cases of human angiostrongyliasis reported worldwide since 2008

Regions	Cases	References
China	65	[17, 18]
Thailand	8	[19, 20]
India	1	[21]
French	1	[22]
Germany	1	[23]
Jamaica	1	[24]

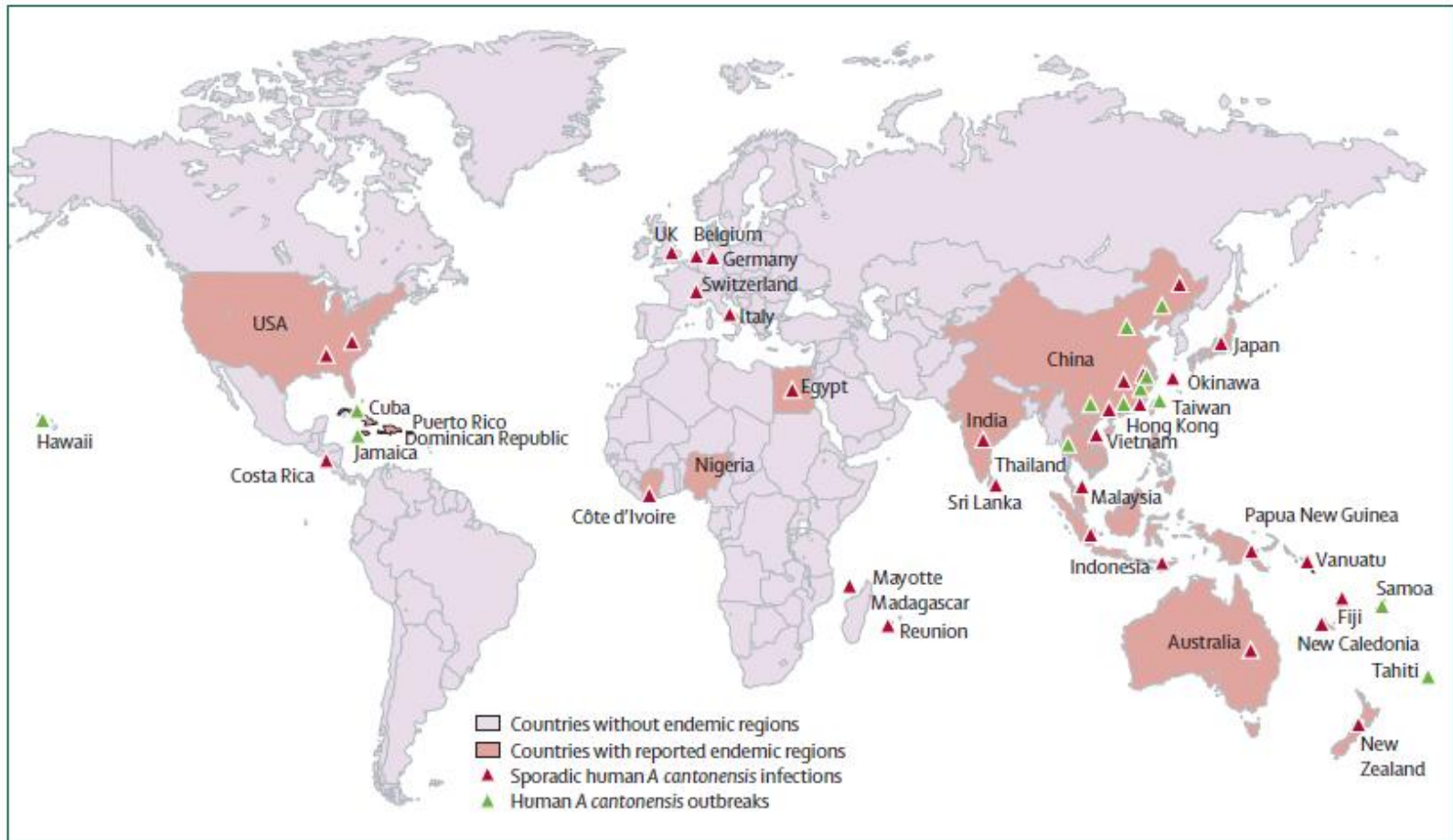
Number of cases in China has increased in recent years. Outbreaks have been reported

Table 2 The outbreaks of human angiostrongliasis have been reported in mainland China since 1997

Years	Regions	Cases	References
1997	Zhejiang	65	[25]
1999	Heilongjiang	2	[26]
1999	Liaoning	3	[26]
2002	Fujian	8	[27]
2002	Fujian	13	[28]
2002	Fujian	8	[29]
2003–2005	Yunnan	28	[30]
2004	Zhejiang	3	[31]
2005–2006	Yunnan	31	[32]
2006	Beijing	160	[33]
2007	Guangdong	6	[34]
2006–2008	Guangdong	32	[17]
2007–2008	Yunnan	33	[18]



Angiostrongylus cantonensis



Wang et al. *Lancet Infect Dis* 2008 10:621-30

Angiostrongylus cantonensis



Countries with autochthonous cases of RLW infection or RLW detected in environmental samples

Australia

Brazil

Cambodia

China

Cuba

Ecuador (outbreak 2008/2009)*

Egypt

Fiji

India

Ivory Coast

Jamaica

Japan

Madagascar

Malaysia

Nigeria

Philippines

Puerto Rico

South Africa (2011) –Rats**

Taiwan

Tahiti

Thailand

US

Vietnam

*Dorta-Contreras AJ, et al.
2011 Arq Neuropsiquiatr;69(3):466-469

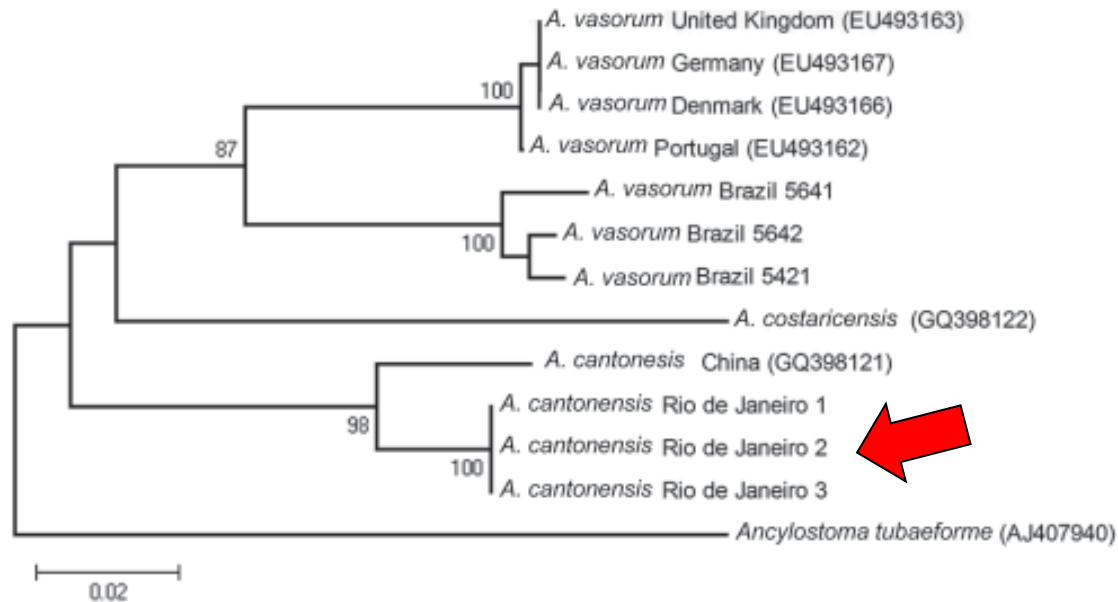
*Pincay, et al. Angiostrongiliasis por Parastrongylus
(Angiostrongylus) cantonensis en Ecuador. Bol Epid (Ecu)
2009;6:25-32.

**Archer CE, Appleton CC, MukaratirwaS, Hope KJ 2011,
Vol. 101, No. 3 SAMJ

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INFECTIOUS DISEASE PREVENTION & PUBLIC HEALTH PARTNERSHIPS • HEALTHY COMMUNITIES • VACCINATION AND CONTROL

Angiostrongylus cantonensis



Phylogenetic tree based on COI gene:

Three *Angiostrongylus* specimens recovered from the pulmonary arteries of a naturally infected Norway rat (*Rattus norvegicus*) from São Gonçalo, Rio de Janeiro, Brazil, 2010.

Angiostrongylus cantonensis

Detection in the environment

Microscopy: Identification of larvae and adult worms

Molecular: Identification by PCR, real-time PCR and/or DNA sequencing analysis

Genetic Targets

18SrRNA gene

Internal Transcribed Spacers (ITS)1 and 2

Cytochrome c oxidase subunit I (COI)



Adult worm from rodent



Stage 3 larva from mollusk

Could dirty genome approach help us identify new targets to improve detection?

