Comparison of *Angiostrongylus* Species Identification Through Morphological and Molecular Methods Using the COX1 Gene as a Marker

Aran Manalang[1], Kittipong Chaisiri[2], Urusa Thaenkham[2], and Paron Dekumyoy[2]

[1]Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA; [2]Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

INTRODUCTION

Angiostrongylus is a parasitic nematode found mostly in the Asia-Pacific region, and causes the disease called angiostrongyliasis. This disease is often associated with eosinophilic meningitis caused by the parasite- *Angiostrongylus cantonensis*- also known as the rat lungworm (PMID: 27225800). The 2 most commonly found species of *Angiostrongylus* found in Thailand are *A. cantonensis* and *A. malaysiensis*. Both species contain a very similar life cycle, but only *A. cantonensis* has been confirmed as a human pathogen (PMID: 27940331). Although there are currently no confirmed cases of *A. malaysiensis* infection in humans, the paramount relatedness between the two species suggests that *A. malaysiensis* may potentially be pathogenic. Therefore, being able to discern between the *Angiostrongylus* species caries great clinical importance.

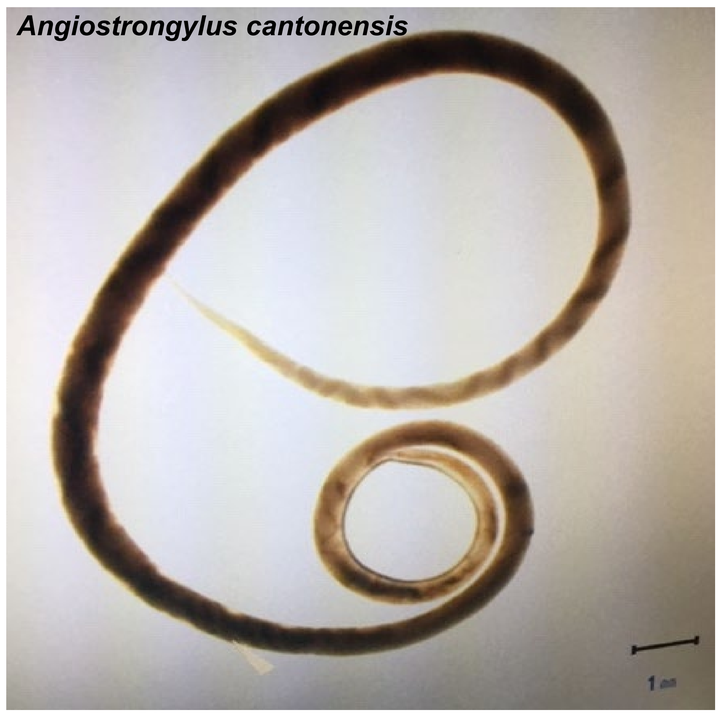
The definitive host of this parasite consist of multiple species of rats in the genus *Rattus* (PMID: 27225800*)*. Whereas, the intermediate host for this parasite are snails and slugs. The parasite matures and lays eggs in the rodents. The eggs hatch into the first-stage larvae (L1) and are excreted from the rodent through their feces. The intermediate host consumes the infected feces, where the larvae will mature and develop into stage-three larvae (L3)- the infective stage. If the rodent consumes the infected intermediate host, the L3 larvae will infect the rodent allowing it to develop into mature adults that is able to reproduce and continue the cycle (PMID: 27225800 & 31287041). Humans are dead-end hosts that usually become infected through the consumption of undercooked- or raw- intermediate hosts such as snails and mollusks that is infected with the L3 larvae (PMID: 27225800).

QUESTION

Will the morphological identification of the two species match with their corresponding molecular analysis?

MATERIALS & METHODS

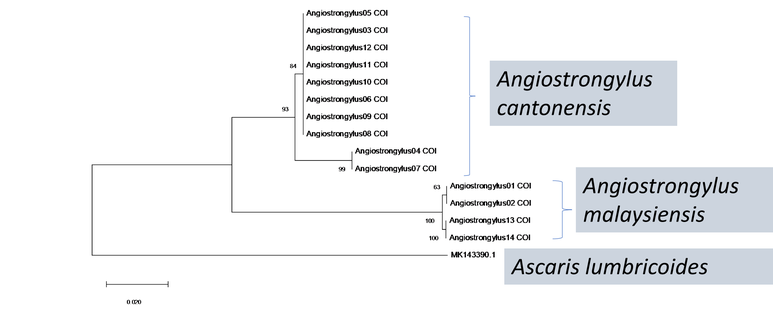
- Fourteen adult worms were obtained from Phayao province which is located in Northern Thailand.
- Morphological identification was performed using two different programs and microscopes.
    1. Olympus Program with stereomicroscope/dissection microscope.
    2. Zen program with inverted microscope
- DNA extracted with Qiagen DNA extraction kit.
- Polymerase chain reaction (PCR) was used to amplify the mitochondrial cytochrome c oxidase subunit 1 (COX1) gene.
    - Amplicon size of about 420-bp.
- DNA sequence editing and analysis was conducted using Bioedit program
    - Pairwise alignment between forward and reverse sequence.
- DNA alignment done with Clustal X program
- Phylogenetic tree created with Mega-X program.



There is a **28.6% mismatch** between phenotypic and genotypic identification of pathogenic rat lung worm *(Angiostrongylus cantonensis)*, from the nonpathogenic *A. malaysiensis species*

amgm@hawaii.edu

RESULTS



Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Angiostrongylus malaysiensis isolate Am4L3219 cytochrome c oxidase subunit 1 (cox1) gene, partial cds; mitochondrial	688	688	100%	0.0	99.73%	KU532149.1
Angiostrongylus malaysiensis isolate Am29L3VIE cytochrome c oxidase subunit 1 (cox1) gene, partial cds; mitochondrial	682	682	100%	0.0	99.47%	KU532154.1
Angiostrongylus malaysiensis isolate Am20L3PHA cytochrome c oxidase subunit 1 (cox1) gene, partial cds; mitochondrial	682	682	100%	0.0	99.47%	KU532162.1
Angiostrongylus malaysiensis isolate Am19L3PHR cytochrome c oxidase subunit 1 (cox1) gene, partial cds; mitochondrial	682	682	100%	0.0	99.47%	KU532161.1
Angiostrongylus malaysiensis isolate AM6 mitochondrion, complete genome	682	682	100%	0.0	99.47%	KT947979.1

Figure 1. Phylogenetic Tree created from the DNA sequences generated from the COX1 gene amplification. The phylogenetic tree was created using Neighbor Joining and Maximum Likelihood method, with a bootstrap value of 1000x. The two methods generated the same tree as shown here. Clades were identified by running the DNA sequence through BLAST. Shown above is the result from one of the samples found in one of the clades that shows that the sequence is 99.73% identical to those found in *Angiostrongylus malaysiensis* indicating that the clade belongs to the *Angiostrongylus malaysiensis. Ascaris lumbricoides* COX1 mitochondrial DNA was acquired from the Genbank and used as an outgroup.

Table 1. Table showing the comparison between the molecular and morphological result The results from *Angiostrongylus* sample 13, 10, 5, and 3 between the two assays contrasted which resulted in a 4/14 (28.6%) mismatch.

Sample	Molecular Identification	Morphological Identification	Village	Match
Angiostrongylus 4	A. cantonensis	A. cantonensis	A	✓
Angiostrongylus 6	A. cantonensis	A. cantonensis	A	✓
Angiostrongylus 7	A. cantonensis	A. cantonensis	A	✓
Angiostrongylus 8	A. cantonensis	A. cantonensis	A	✓
Angiostrongylus 9	A. cantonensis	A. cantonensis	A	✓
Angiostrongylus 1	A. malaysiensis	A. malaysiensis	B	✓
Angiostrongylus 2	A. malaysiensis	A. malaysiensis	C	✓
Angiostrongylus 12	A. cantonensis	A. cantonensis	C	✓
Angiostrongylus 11	A. cantonensis	A. cantonensis	C	✓
Angiostrongylus 14	A. malaysiensis	A. malaysiensis	C	✓
Angiostrongylus 13	A. malaysiensis	A. cantonensis	C	⊘
Angiostrongylus 10	A. cantonensis	A. malaysiensis	A	⊘
Angiostrongylus 5	A. cantonensis	A. malaysiensis	A	⊘
Angiostrongylus 3	A. cantonensis	A. malaysiensis	C	⊘

CONCLUSIONS

Further refinement and research are needed to achieve a 0% mismatch to unequivocally differentiate the two species of *Angiostrongylus*.

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