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Philippine Survey of Nematode Parasite Infection and Load in the Giant African Snail *Achatina fulica* indicate *Angiostrongylus cantonensis* infection in Mindanao

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ABSTRACT

Achatina fulica is a ubiquitous land snail commonly found throughout the Philippines. As a generalist feeder and being able to survive in a wide range of habitat types and conditions, the snail can easily establish itself in a new area after introduction. It also acts as host to a variety of parasites, including nematodes, which may accidentally infect humans. In this study, *A. fulica* individuals from 13 areas in the Philippines were sampled and analyzed for nematode infection rate and load. Of the 393 individuals sampled, 80 (20%) were found to be infected, with 5049 nematodes isolated. The infection rates and parasite load were highly variable. Overall, the parasite load ranges from 1 to 867 per snail. Representative nematodes from *A. fulica* from Plaridel (n=8) and Davao City (n=26) in Mindanao were subjected to DNA extraction, PCR amplification, and sequencing of the SSU rRNA gene, which is the universal barcode for nematodes. Sequences successfully matched with the dog lungworm *Oslerus osleri* for the Plaridel nematodes and the rat lungworm *Angiostrongylus cantonensis* for the Davao City nematodes, respectively. The latter is known to infect humans and can cause eosinophilic meningoencephalitis. This study presents the first report of *A. cantonensis* in *A. fulica* from Mindanao and raises a public health concern.

Keywords: *Achatina fulica,* nematode, Philippines, SSU rRNA, *Oslerus osleri*, *Angiostrongylus cantonensis*

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INTRODUCTION

The Giant African Land Snail, *Achatina fulica* (Family Achatinidae), is a ubiquitous snail with characteristic reddish brown markings on the shell (Jarrett 1931, Raut and Barker 2002) and has the ability to adapt to a wide range of environmental conditions (Cobbinah and others 2008). Its habitat and diet include a wide variety of plant species (Jarrett 1931, Raut and Barker 2002). *A. fulica* originated from eastern coastal Africa but has spread throughout Asia and the Pacific, including the Philippines, during World War II. *A. fulica* was introduced to these regions by the Japanese to be used as an alternative food source (Alicata 1966, Latonio 1971).

A. fulica is capable of carrying large numbers of parasitic nematodes that may infect humans (Kliks and Palumbo 1992). The snail is a known intermediate host of the rat lungworm *Angiostrongylus cantonensis* (Tsai and others 2004, Cobbinah and others 2008, Zhang and others 2009, Fontanilla and Wade 2012, Constantino-Santos and others 2014). *A. cantonensis* infection causes eosinophilic meningitis in humans, and outbreaks of the disease have been associated with exposure to infected *A. fulica* (Tsai and others 2004). Humans may become infected by *A. cantonensis* via consumption or contact with an infected snail. Consumption of *A. fulica* is quite common due to its high protein, iron, and calcium content (Tsai and others 2003, Cobbinah and others 2008). Infection by contact with *A. fulica* was documented in Taiwan, especially among children playing with the snail (Tsai and others 2004). Apart from *A. cantonensis, A. fulica* has also been shown to harbor *Rhabditis* sp. (Seehabutr 2005), the cat lungworm *Aelurostrongylus abstrusus* (de Andrade-Porto and others 2012), and the dog lungworm *Ancylostoma caninum* (Constantino-Santos and others 2014).

Numerous studies in the Philippines have targeted gastropods, including *A. fulica*, to determine the presence of a specific nematode, *A. cantonensis*, but these were limited to Luzon (Salazar and Cabrera 1969, Westerlund and Chamberlain 1969, Latonio 1971, Fontanilla and Wade 2008). A recent study by Constantino-Santos and others (2014) demonstrated the presence of two medically important nematodes, *A. cantonensis* and *A. caninum*, and 12 other unidentified nematodes in *A. fulica* populations found in Metropolitan Manila. They used the SSU rRNA gene to identify the nematodes or determine their closest match.

In this study, a survey of the nematode parasite load and infection rate in *A. fulica* populations was conducted in 13 different parts of the Philippines. Furthermore, nematodes in the Mindanao populations, which were surveyed for the first time, were identified through DNA sequencing of the SSU rRNA gene.

MATERIALS AND METHODS

Samples of *A. fulica* were collected from 13 different areas in the Philippines (Figure 1). The sites chosen were representative urban areas from Luzon, Visayas, and Mindanao where *A. fulica* is known to be common. Purposive sampling was done to collect at least 30 adult-sized snails in habitats where they could be found, i.e., gardens and roadsides. Each live snail was then brought to the lab, cut into small pieces, and digested overnight in Ash's (1970) digestive fluid containing 0.7% pepsin in 0.5% HCl. Individual nematodes were collected and counted. The prevalence of nematode infection for each site was computed by taking the percentage of snails infected with nematodes. The parasite load range of *A. fulica* for each site was also determined.

Preliminary identification of nematodes was performed using the SSU rRNA gene, which is the standard molecular barcode for soil nematodes (Floyd and others 2002, Fontanilla and Wade 2008). Snails from Davao City, Davao and Plaridel, Misamis Occidental, both from Mindanao, were selected as source of nematodes for molecular identification. Nematodes were selected at random and stored at -20°C prior to use. Genomic DNA was obtained from these nematodes via NaOH lysis method modified from Floyd and others (2002). The nematodes were placed in microcentrifuge tubes with 20 µL of 0.25 M NaOH, then centrifuged for a few seconds at 16,276 x g to ensure complete submersion. They were incubated for 5 h,

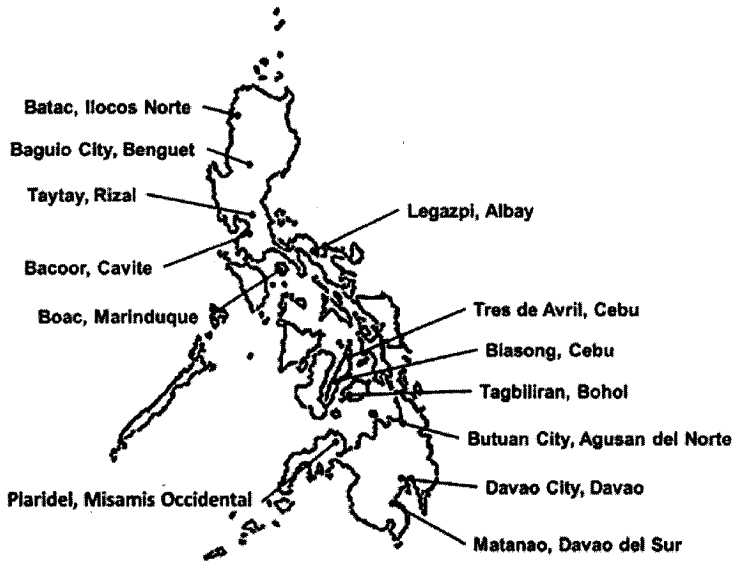

Figure 1. Areas sampled in this study.

after which the samples were heated at 95°C for 3 min, followed by cooling at room temperature and another round of centrifugation for a few seconds at 16,276 x g. Then, 4 μL of 1.0 M HCl, 10 μL of 0.5 M Tris-HCl, and 5 μL 2% of Triton X-100 were added. The samples were then centrifuged for a few seconds at 16,276 x g, heated at 95°C for 3 min, and cooled at room temperature. The samples were stored at -20°C prior to use.

The SSU rRNA gene was subsequently amplified through PCR. The primers used in this study were as follows: SSU_F_07 (sense) 5' – AAAGATTAAGCCATGCATG – 3' and SSU_R_09 (anti-sense) 5' – AGCTGGAATTACCGCGGCTG – 3' (Blaxter and others 1998). These primers produce a PCR product of approximately 480 bp from the 5' end of the SSU rRNA gene.

A total volume of 50 μL for the PCR mix was prepared and consisted of the following components: 5 μL PCR buffer with 1.5 mM $MgCl_2$, 1.0 μL 10 mM dNTP, 2.5 μL 10 μM of each primer, 10 μL Q buffer (Qiagen, Netherlands), 0.25 μL 1.25 U Taq (Roche™, USA), and 4 μL (5-20 ng/μL) DNA sample. The amplification was performed using the Labnet MultiGene™ thermocycler. Conditions for the PCR run were set as follows: 94°C for 3 min and 43 cycles of 94°C for 30 s, 45°C for 30 s, and 65°C for 1 min, with the final extension at 72°C for 5 min. The PCR products were visualized in 1% agarose gel (1.0 g agarose in 100 mL TBE buffer [1.1 M Tris, 900 mM Boric acid, 25 mM EDTA, pH 8.3]) with 1.0 0 μL of 10 mg/mL ethidium bromide under UV illumination. Each PCR band was cut from the gel using a sterile scalpel blade.

Each excised gel was placed in a 1.5 mL microcentrifuge tube and weighed. The Qiagen™ Gel Extraction Kit (USA) was then used to purify the PCR products by removing the agarose. The purified PCR products were sent to Philippine Genome Center at the National Institute of Molecular Biology and Biotechnology in University of the Philippines, Diliman and 1st Base, Malaysia, for sequencing. The anti-sense strands were sequenced using the R09 primer via capillary sequencing, also known as Sanger sequencing, using the Applied Biosystems 3730xl DNA Analyzer.

STADEN package version 1.5.3 (Staden and others 2000) was initially used to assemble and align the DNA sequences to check for ambiguous nucleotide sites. The sequences were then manually aligned in the BioEdit Sequence Alignment Editor 7.0.9.0 (Hall 1999). Each sequence was then subjected to Basic Local Alignment Search Tool (BLAST) (Altschul and others 1990) to identify the closest match of each individual.

RESULTS AND DISCUSSION

All areas sampled were found to have infected snails except for Batac, Ilocos Norte. Infection rates for infected populations, however, varied, ranging from 3% to 39%. Even the parasite load of infected snails exhibited high variability, ranging from 1 to 867 nematodes per snail. Davao City, in particular, had the highest infection rate and total number of parasites (Table 1). The variation in parasite load could be a function of the relative age of the snail. Sithithaworn and others (1991) found a positive correlation between the age of the snail based on its shell size and the parasite load; they noted a mean parasite load of 5478 per infected snail in the oldest snail group (>6.60 cm shell length). On the other hand, the variation in rate of infection across the different *A. fulica* populations may be due to the patchy distribution of both the parasite and snail host. For instance, Bisseru (1971) observed high variation of infection rates in *A. fulica* even within a small geographic area in West Malaysia, with two populations exhibiting no infection. The prevalence of the parasite is subject to the availability of the hosts, both definitive and intermediate, for the parasite to complete its life cycle.

Eight nematode samples from Plaridel were successfully subjected to direct sequencing of the 5' end of the SSU rRNA gene and gave higher than 99.5% identity with Nematoda sp. Fontanilla (GenBank EF514918) and *Oslerus osleri* (GenBank AY295812) based on BLAST results. The BLAST results for the Plaridel nematodes

Table 1. Infection rates and parasite load of *A. fulica* specimens from 13 sites in the Philippines

Site	Sample size	Number of infected snails (Percentage)	Total number of nematodes (Parasite Load Range)
Bacoor, Cavite	30	3 (10%)	53 (2-49)
Baguio City, Benguet	30	4 (13%)	82 (1-68)
Batac, Ilocos Norte	30	0 (0%)	0 (0)
Biasong, Cebu	30	4 (13%)	10 (1-5)
Legazpi, Albay	30	8 (27%)	792 (1-743)
Boac, Marinduque	30	11 (37%)	184 (2-160)
Butuan City, Agusan del Norte	30	5 (17%)	1478 (5-867)
Davao City, Davao	30	11 (37%)	2300 (1-791)
Matanao, Davao del Sur	30	2 (7%)	8 (2-6)
Plaridel, Misamis Occidental	33	13 (39%)	74 (1-27)
Tagbilaran City, Bohol	30	10 (33%)	43 (1-18)
Taytay, Rizal	30	8 (24%)	24 (1-7)
Tres de Avril, Cebu	30	1 (3%)	1 (1)
Total	393	80 (20%)	5049 (0-867)

are summarized in Table 2. Following the 99.5% threshold value proposed by Floyd and others (2002) in identifying nematodes using the SSU rRNA gene, these nematodes are therefore in the same molecular operational taxonomic unit (MOTU) as *Oslerus osleri*.

O. osleri is previously classified as *Filaroides osleri*. It is a metastrongyle parasite with worldwide distribution and is a widely occurring tracheal parasite capable of causing respiratory disease in domestic and wild canids such as dogs. It is transmitted by direct contact and oral ingestion of the first-stage larva (Foreyt and Foreyt 1981, Outerbridge and Taylor 1998).

The life cycle of this nematode does not involve an intermediate host (Outerbridge and Taylor 1998). Mucociliary apparatus carries the eggs and the infective first-stage larva from the tracheal bifurcation to the oropharynx, where they are either swallowed and shed in the feces or shed in the saliva. The primary sources of the parasite are the asymptomatic dogs (Outerbridge and Taylor 1998). Following infection, the first-stage larvae penetrate the mucosa of the gastrointestinal tract. It then travels to the right side of the heart via lymphatics or the hepatic venous circulation where the larva migrates to the lungs via pulmonary arteries (Yao and others 2011). Development from the first-stage larva to adult occurs in the respiratory tract where tracheobronchial nodules are formed, thus completing the life cycle (Clayton and Lindsay 1979). Maternal grooming is assumed to be the major transmission route in domestic dogs; for free-ranging canids, the regurgitative feeding of the young by parents appeared to be the major means of infection (Clayton and Lindsay 1979, Bowman 2009).

The presence of *O. osleri* in *A. fulica* in Plaridel, Misamis Occidental could be a result of accidental infection as the snail is not the definitive or final host of the parasite and may have only been shed via the feces by an infected dog. A previous study by Constantino-Santos and others (2014) also detected the presence of worms most similar to *O. osleri* (99.1% identity) and *O. rostratus* (99.5%–99.7% identity) in *A. fulica* populations in Metro Manila, Philippines. Nevertheless, detection of *O. osleri* in Plaridel indicated that *A. fulica* could serve as another route of infection for humans, especially to those who come in contact with these snails.

Twenty six nematode samples from Davao City had the 5' end of their SSU rRNA gene successfully sequenced and identified as *Angiostrongylus cantonensis* (GenBank GQ181114) based on BLAST results and Floyd's 99.5% threshold value, with five of them having 100% identity with *A. cantonensis*, as shown in Table 2.

Table 2. Identity of nematodes from *Achatina fulica* in Plaridel, Misamis Occidental and Davao City, Davao based on BLAST

Specimen Code	Accession Number	Best Match	% Similarity	Gaps
Plaridel, Misamis Occidental				
15-2A	EF514918	Nematoda sp. Fontanilla D17-D1	446/446(100%)	0/446(0%)
	AY295812	*Oslerus osleri*	444/446(99.6%)	0/446(0%)
15-2B	EF514918	Nematoda sp. Fontanilla D17-D1	406/406(100%)	0/406(0%)
	AY295812	*Oslerus osleri*	405/406(99.8%)	0/406(0%)
15-3A	EF514918	Nematoda sp. Fontanilla D17-D1	423/423(100%)	0/423(0%)
	AY295812	*Oslerus osleri*	421/423(99.5%)	0/423(0%)
15-3B	EF514918	Nematoda sp. Fontanilla D17-D1	416/416(100%)	0/416(0%)
	AY295812	*Oslerus osleri*	414/416(99.5%)	0/416(0%)
15-5A	EF514918	Nematoda sp. Fontanilla D17-D1	387/388(99.7%)	0/388(0%)
	AY295812	*Oslerus osleri*	386/388(99.5%)	0/388(0%)
15-5B	EF514918	Nematoda sp. Fontanilla D17-D1	420/420(100%)	0/420(0%)
	AY295812	*Oslerus osleri*	418/420(99.5%)	0/420(0%)
15-13	EF514918	Nematoda sp. Fontanilla D17-D1	422/422(100%)	0/422(0%)
	AY295812	*Oslerus osleri*	420/422(99.5%)	0/422(0%)
22-4	EF514918	Nematoda sp. Fontanilla D17-D1	420/421(99.8%)	0/421(0%)
	AY295812	*Oslerus osleri*	418/421(99.3%)	0/421(0%)
Davao City, Davao				
DV1	GQ181114	*Angiostrongylus cantonensis*	428/429(99.8%)	0/429(0%)
DV3	GQ181114	*Angiostrongylus cantonensis*	439/439(100%)	0/439(0%)
DV4	GQ181114	*Angiostrongylus cantonensis*	439/439(100%)	0/439(0%)
DV5	GQ181114	*Angiostrongylus cantonensis*	428/429(99.8%)	0/429(0%)
DV6	GQ181114	*Angiostrongylus cantonensis*	438/439(99.8%)	0/439(0%)
DV7	GQ181114	*Angiostrongylus cantonensis*	434/436(99.5%)	0/436(0%)
DV3A	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3B	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3C	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3D	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3E	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3F	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3G	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3H	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3I	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3J	JN663725	*Angiostrongylus cantonensis*	460/460(100%)	0/460(0%)
DV3K	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3L	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3N	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3O	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3Q	JN663725	*Angiostrongylus cantonensis*	460/460(100%)	0/460(0%)
DV3S	JN663725	*Angiostrongylus cantonensis*	460/460(100%)	0/460(0%)
DV3T	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3X	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3Y	JN663725	*Angiostrongylus cantonensis*	466/467(99.8%)	1/467(0.2%)
DV3AA	JN663725	*Angiostrongylus cantonensis*	468/469(99.8%)	1/469(0.2%)

This study demonstrates that *A. fulica* in Davao facilitates the spread of *A. cantonensis*. The absence of *A. cantonensis* in Plaridel, on the other hand, is not unexpected. In a study by Bisseru (1971), 27 sites in West Malaya were surveyed for *A. cantonensis* from *A. fulica*, and two of these sites did not even yield any parasite. For instance, Padang Besar in Perlis had 0% infection rate from 100 snails sampled, whereas Alor Star, Kedah, which was 65 km away, yielded 28.5% infection rate from 70 snails. The snail intermediate hosts, the rat definitive hosts, or even the parasites, have a patchy distribution themselves; most likely, only those snails found in areas with rats that harbored the parasite were the ones infected (Fontanilla and Wade 2012).

Cases of *A. cantonensis* infection in rodents and molluscs have been documented in the Philippines; however, most of these were found in several provinces in Luzon (Salazar and Cabrera 1969, Westerlund and Chamberlain 1969, Garcia 1979, Antolin and others 2006) and one area in the Visayas (Guerrero and Guerrero 1972). This is the first reported case of *A. cantonensis* in Mindanao in either the snail intermediate host or the rat definitive host. The presence of *A. cantonensis* in Mindanao is not surprising considering the distribution of rats and *A. fulica* all over the Philippines. This information, however, is significant from a public health perspective.

These nematodes cause eosinophilic meningitis or meningoencephalitis (Wan and Weng 2004). They are now generally recognized as the causative agent of human eosinophilic meningitis, also called angiostrongyliasis (Panha 1988), which is characterized by inflammation of the meninges in the human brain and the presence of higher levels of eosinophils in the cerebral spinal fluid (Senanayake and others 2003).

A three-year study done by Latonio (1971) focused on four cases of eosinophilic meningoencephalitis and two cases of myeloencephalitis symptom-complex from patients in the Philippines. He reasoned that infection might not be due to direct consumption, since *A. fulica* is not normally consumed by Filipinos. Rather, it is possibly due to the prevalence of *A. fulica* among edible plants, which could have been contaminated by the snail with *A. cantonensis*, either through their feces or mucus, before they were consumed by humans (Wallace and Rosen 1969, Marquardt and others 2000). Alternatively, handling infected snails, particularly by children who play with them, could have been another possible route of infection (Wan and Weng, 2004).

Occurrence of *A. cantonensis* in Canton, China was first described by Chen (1935) in rats. Rodents are considered its definitive host (e.g., *Rattus rattus*, *R. norvegicus*) whereas intermediate hosts include snails (e.g., *Achatina fulica, Pomacea*

canaliculata) and slugs (e.g., *Imerinia plebeia, Leavicaulis alte*) (Bartschi and others 2003).

The basic life cyle of *A. cantonensis* involves a definitive mammalian host and an intermediate molluscan host. The adult worms live in the pulmonary arteries of their definitive hosts, where the females also lay their eggs. These hatch into first-stage larvae, which are then transmitted to the rats' feces via the trachea and gastrointestinal tract. These larvae enter their intermediate hosts, such as molluscs, through ingestion of the excrement, wherein they turn into third-stage larvae after two successive molts (Lee and Yen 2005). An infected mollusc can carry a highly variable number of second– and third–stage juveniles depending on the degree of infection (Caldeira and others 2007). These third-stage larvae infect the rat host through consumption of the intermediate host. The larvae migrate to the central nervous system where further development occurs until they reach adulthood. From there, they then return to the pulmonary arteries where they undergo sexual maturation (Qvarnstrom 2007).

Humans, upon ingestion of these molluscs in their raw forms, become accidental hosts, manifesting the infection as eosinophilic meningitis (Senanayake and others 2003). The third-stage larvae, similar to what occurs in rodents, migrate to the central nervous system, which consists of the brain and spinal cord tissues. However, these larvae often remain in the central nervous system of human hosts; nevertheless, rare cases exist wherein they continue migration to the lungs. Their presence in the brain and spinal cord causes tissue damage and subsequent inflammation (Qvarnstrom 2007).

To prevent human infection, the most effective method is to educate people to maintain good sanitation in food preparation areas, not to eat raw or undercooked snails, and to avoid eating raw vegetables that may harbor inconspicuous or juvenile snails or slugs in regions where *A. cantonensis* is present (Yang and others 2013). As Davao *A. fulica* snails carry the said parasite, it is important to heighten the awareness of people in the area of the possibility of infection through consuming raw vegetable crops associated with the snails or playing or handling these snails. Moreover, control and monitoring of various intermediate hosts, in this case *A. fulica*, and definitive hosts in areas of epidemiological relevance should be undertaken to lessen the risk to humans.

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REFERENCES

Alicata J. 1966. The presence of *Angiostrongylus cantonensis* in the islands of the Indian Ocean and probable role of the giant African snail, *Achatina fulica*, in the dispersal of the parasite to the Pacific islands. Canadian Journal Zoology 44: 1041-1049.

Altschul S.F., Gish W., Miller W., Myers E.W., Lipman D.J. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403-410.

Anderson R.C. 2000. Nematode parasites of vertebrates: their development and transmission. 2nd ed. Wallingford: CABI. 672 p.

Antolin M.M., Joshi R.C., Sebastian L.S., Marquez L.V., Duque U.G. 2006. Endo- and ecto parasites of the Philippine rice field rat, *Rattus tanezumi* Temminck, on PhilRice farms. International Rice Research Notes 31: 26-27.

Ash L.R. 1970. Diagnostic morphology of the third-stage larvae of *Angiostrongylus cantonensis, Angiostrongylus vasorum, Aelurostrongylus abstrutus,* and *Anafilaroides rostratus* (Nematoda: Metastrongyloidea). Journal of Parasitology 56: 249-253.

Bartschi E., Bordmann G., Blum J., Rothen M. 2003. Eosinophilic meningitis due to *Angiostrongylus cantonensis* in Switzerland. Infection 32: 116-118.

Bisseru B. 1971. The prevalence of *Angiostrongylus cantonensis* larvae collected from the giant African snail, *Achatina fulica*, in West Malaysia and Singapore. Southeast Asian Journal for Tropical Medicine and Public Health 2: 523-526.

Blaxter M.L., De Ley P., Garey J.R., Liu L.X., Scheldeman P., Vierstraete A., Vanfleteren J.R., Mackey L.Y., Dorris M., Frisse L.M., Vida J.T., Thomas W.K. 1998. A molecular evolutionary framework for the phylum nematoda. Nature 392(6671): 71-75.

Bowman D. 2009. Georgis' parasitology for veterinarians. 9th ed. Missouri: Saunders/ Elsevier. 451 p.

Caldeira R., Mendonca C., Goveia C., Lenzi H., Graeff-Teixeira C., Lima W., Mota E., Percoa I., de Medeiros A., Carvalho O. 2007. First record of molluscs naturally infected with *Angiostongylus cantonensis* (Chen, 1935) (Nematoda: Metastringylidae) in Brazil. Memorias do Instituto Oswaldo Cruz 102: 887-889.

Chen H. 1935. A new pulmonary nematode of rats, *Pulmonema cantonensis* ng, nsp from Canton [in French]. Annals of Tropical Medicine and Parasitology 13: 312-317.

Clayton H., Lindsay F. 1979. *Filaroides osleri* infection in the dog. Journal of Small Animal Practice 20: 773–782.

Cobbinah J.R., Vink A., Onwuka B. 2008. Snail farming: production, processing and marketing. 1st ed. Wageningen, Netherlands: Agromisa Foundation. 79 p.

Constantino-Santos D.M.A., Basiao Z.U., Wade C.M., Santos B.S., Fontanilla I.K.C. 2014. Identification of *Angiostrongylus cantonensis* and other nematodes using the SSU rDNA in *Achatina fulica* populations of Metro Manila. Tropical Biomedicine 31: 327-335.

de Andrade-Porto S.M., de Souza K.C.P., Cardenas M.Q., Roque R.A., Pimpao D.M., Araujo C.S., de Oliveira Malta J.C. 2012. Occurrence of *Aelurostrongylus abstrusus* (Railiet, 1898) larvae (Nematoda: Metastrongylidae) infecting *Achatina* (*Lissachatina*) *fulica* Bowdich, 1822 (Mollusca: Gastropoda) in the Amazon region. Acta Amazonica 42: 245-250.

Floyd R., Abebe E., Papert A., Blaxter M. 2002. Molecular barcodes for soil nematode identification. Molecular Ecology 11: 839-850.

Fontanilla I.C., Wade C.M. 2008. The small subunit (SSU) ribosomal (r) RNA gene as a genetic marker for identifying infective 3rd juvenile stage *Angiostrongylus cantonensis*. Acta Tropica 105: 181-186.

Fontanilla I.C., Wade C.M. 2012. First report of *Angiostrongylus cantonensis* in the giant African land snail *Achatina fulica* in French Polynesia detected using the SSU rRNA gene. Tropical Biomedicine 29: 642-645.

Foreyt W., Foreyt K. 1981. Attempted transmission of *Oslerus (Oslerus) osleri (=Filaroides osleri)* from coyotes to domestic dogs and coyotes. The Journal of Parasitology 67: 284-286.

Garcia E.G. 1979. *Angiostrongylus cantonensis* in the Philippines: a review. In: J.H. Cross. Angiostrongyliasis in Eastern Asia and Australia. Taipei: NAMRU-2-SP-44. p 53-56.

Guerrero L.A., Guerrero R.I. 1972. *Angiostrongylus cantonensis* in commercial rats in Dumaguete City, Negros Oriental. Acta Medica Philippina 8: 33-35.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95-98.

Jarrett V.H.C. 1931. The spread of the snail *Achatina fulica* to South China. Hong Kong Naturalist 2: 73-76.

Kliks M.M., Palumbo NE. 1992. Eosinophilic meningitis beyond the Pacific Basin: the global dispersal of a peridomestic zoonosis caused by *Angiostrongylus cantonensis*, the nematode lungworm of rats. Social Science Media 34: 199-212.

Lai C., Yen C., Chin C., Chung H., Kuo H., Lin H. 2007. Eosinophilic meningitis caused by *Angiostrongylus cantonensis* after ingestion of raw frogs. American Journal Tropical Medicine and Hygiene 76: 399-402.

Latonio A.A. 1971. The giant African snail, *Achatina fulica*: a new threat to public health. Transactions of the Royal Society Tropical Medicine and Hygiene 65: 22.

Lee J., Yen C. 2005. Protease secreted by the infective larvae of *Angiostrongylus cantonensis* and its role in the penetration of mouse intestine. The American Journal of Tropical Medicine and Hygiene 72: 831-836.

Marquardt, W.C., Demaree, R.S., Grieve, R.B. 2000. Parasitology and vector biology. 2nd ed. San Diego, California: Academic Press. 702 p.

Outerbridge C., Taylor S. 1998. *Oslerus osleri* tracheobronchitis: treatment with ivermectin in 4 dogs. Canadian Veterinary Journal 39: 238-240.

Panha S. 1988. Natural infection of the rat lungworm *Angiostrongylus cantonensis* in a Thai edible land snail, *Hemiplecta distincta*. Journal of the Science Society of Thailand 14: 233-239.

Qvarnstrom Y., Sullivan J.J., Bishop H.S., Hollingsworth R., da Silva A.J. 2007. PCR-based detection of *Angiostrongylus cantonensis* in tissue and mucus secretions from molluscan hosts. Applied and Environmental Microbiology 73: 1415-1419.

Raut S.Y., Barker G.M. 2002. *Achatina fulica* Bowdich and other Achatinidae as pests in tropical agriculture. In: Barker, G.M. (ed.) Molluscs as Crop Pests. Hamilton: New Zealand: CABI. p 55-114.

Salazar N.P., Cabrera B.D. 1969. *Angiostrongylus cantonensis* in rodent and molluscan hosts in Manila and suburbs. Acta Medica Philippina 6: 20-25.

Seehabutr V. 2005. Nematodes in alimentary tracts of giant African snails (*Achatina fulica*) in Thailand. Kamphaengsaen Academy Journal 3: 37-41.

Senanayake S., Pryor D., Walker J., Konecny P. 2003. First report of human angiostrongyliasis acquired in Sydney. Medical Journal of Australia 179: 430-431.

Sithithaworn P., Brockelman W.Y., Brockelman C. 1991. Transmission of *Angiostrongylus cantonensis* through the giant African snail *Achatina fulica*: an experimental study. Southeast Asian Journal of Tropical Medicine and Public Health 22 Supplement: 200-205.

Staden R., Beal K., Bonfield J. 2000. The Staden package, 1998. Methods in Molecular Biology 132: 115-130.

Tsai H.C., Liu Y.C., Kunin C.M., Lai P.H., Lee S.S., Chen Y.S., Wann S.R., Lin W.R., Huang C.K., Ger L.P., Lin H.H., Yen M.Y. 2003. Eosinophilic meningitis caused by *Angiostrongylus cantonensis* associated with eating raw snails: correlation of brain Magnetic Resonance Imaging scans with clinical findings. American Journal of Tropical Medicine Hygiene 68: 281–285.

Tsai H., Lee S.S., Huang C., Yen C., Chen E., Liu Y. 2004. Outbreak of eosinophilic meningitis associated with drinking raw vegetable juice in southern Taiwan. American Journal of Tropical Medicine and Hygiene 71: 222-226.

Wallace G.D., Rosen L. 1969. Molluscan hosts of *Angiostrongylus cantonensis* on Pacific Islands. The American Journal of Tropical Medicine and Hygiene 18(2): 206-216.

Wan K., Weng W. 2004. Eosinophilic meningitis in a child raising snails as pets. Acta Tropica 90: 51-53.

Westerlund N.C., Chamberlain M. 1969. Further observations on *Angiostrongylus cantonensis* in the Philippines. Acta Medica Philippina 6: 3-11.

Yang T., Wu Z., Lun Z. 2013. The Apple Snail *Pomacea canaliculata*, a novel vector of the rat lungworm, *Angiostrongylus cantonensis*: its introduction, spread, and control in China. Hawai'i Journal of Medicine & Public Health 72: 23-25.

Yao C., O'Toole D., Driscoll M., McFarland W., Fox J., Cornish T., Jolley W. 2011. *Filaroides osleri (Oslerus osleri)*: two case reports and a review of canid infections in North America. Veterninary Parasitology 179(1-3): 123-9.

Zhang L.H., Chen J.L., Dong W.R. 2009. Analysis of a survey of the infection of *Achatina fulica* with *Angiostrongylus cantonensis* in Foshan, Guangdong Province. Acta Parasitologica et Medica Entomologica Sinica 16: 244-246.

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