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Communication

[*Comunicação*]

Prevalence of *Angiostrongylus cantonensis* and *Angiostrongylus costaricensis in Achatina fulica* snails in the municipality of São Bernardo do Campo (SP, Brazil)

[*Prevalência de Angiostrongylus cantonensis e Angiostrongylus costaricensis em caramujos Achatina fulica na cidade de São Bernardo do Campo (SP, Brasil)*]

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Both *Angiostrongylus cantonensis* and *Angiostrongylus costaricensis* are parasitic nematodes belonging to the superfamily *Metastrongyloidea* that reside in rodents and use gastropods, more commonly known as snails and slugs, as intermediate hosts (Pien and Pien, 1999; Espírito-Santo *et al*., 2013; Giannelli *et al*., 2016). Rats (*Rattus spp.*) are the definitive hosts and transmit their larvae through their feces (Pien and Pien, 1999; Barratt *et al*., 2016). The snail *Achatina fulica*, known as the giant African land snail, is an intermediate host playing an important role in the dispersion of *A. cantonensis* and *A. costaricensis*, zoonotic pathogens that cause, respectively, eosinophilic meningoencephalitis and abdominal angiostrongyliasis in humans (Klikst and Palumbo, 1992; Graeff-Teixeira, 2007; Morassutti *et al*., 2014). *A. cantonensis* has spread from Southeast Asia to the South Pacific, Africa, India, the Caribbean, and, recently, to Australia and the Americas, probably as a consequence of global warming or other environmental factors, such the efficient dispersion of ship-borne rats and the diversity of its intermediate hosts (Pien; Pien, 1999; Barrett *et al*., 2016). In its turn, *A. costaricensis* has been reported from Southern United States to Northern Argentina and in Brazil there is an endemic area in the Southern states of Paraná, Santa Catarina and Rio Grande do Sul (Silva *et al*., 2013). The relatively restricted range of *A. costaricensis* is probably related to the limited distribution of its preferred definitive host, the hispid cotton rat (*Sigmodon hispidus*), which is usually found only in Southern United States and parts of Central and South America (Barratt *et al*., 2016).

The first record of molluscs naturally infected with *A. cantonensis* in Brazil, including *A. fulica*, was done in the state of Espírito Santo, Brazil, in 2007 (Caldeira *et al*., 2007), and the first report of eosinophilic meningitis caused by *A. cantonensis* in the city of São Paulo, Brazil, dates back to 2013 (Espírito-Santo *et al*., 2013). *A. fulica* snail is currently present in 24 out of 26 Brazilian States and in the Federal District. Such explosive dissemination illustrates the current concern with global changes favouring dissemination of infectious diseases. Dense populations of *A. fulica* are problematic to human populations as well as menaceful to gardens and small crops, and act in the transmission of the aforementioned zoonotic diseases and other parasitosis of veterinary importance (Graeff-Teixeira, 2007). In such context, the aim of this investigation was to collect *A. fulica* snails (n= 90) from different regions of the municipality of São Bernardo do Campo (SP, Brazil) from January to June 2018 for molecular detection of *A. cantonensis* or *A. costaricensis.*

The snails were collected, preferably on rainy days, from 8 different regions (Rudge Ramos, Baeta Neves, Planalto, Demarchi, Dos Alvarengas, Batistini, Montanhão, Rio Grande)

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of this municipality. They were then packed and sent to the Laboratory of Molecular Biology of the University Cruzeiro do Sul, where the analyses were performed. According to the size of the snails, molluscs from the same location were pooled (n= 25, Table 1) and then digested. Digestion was performed according to the protocol proposed by Wallace and Rosen, followed by the Baermann-Moraes sedimentation method (Caldeira *et al*., 2007). After sedimentation, the material was analyzed under a stereomicroscope for nematode larvae and the larvae from each collection point (pool) were counted and submitted to molecular studies. DNA extraction and purification was performed using the Qiagen kit Dneasy Blood & Tissue (Invitrogen, Carlsbad, CA, USA), according to manufacturer´s instructions. Summarily, 180 μL of ATL buffer and 20 μL of proteinase K from the kit were added to the sample, mixed by vortex and after overnight incubation at 56°C. The polymerase chain reaction (PCR) technique was carried out using the 150pmol of each primer (NC1 - 5´ ACGTCTGGTTCAGGGTTGTT 3´ and NC2 - 5´ TTAGTTTCTTTTCCTCCGT CT 3´, Invitrogen, Carlsbad, CA, USA), 1U Taq DNA Polymerase and 20μL of the DNA extracted from each pooled sample. These primers were designed by Gasser (1993) and anchored in the conserved regions in the final portion of the subunit 5.8S and the initial portion of subunit 28S. Amplification was performed with a GeneAmp 9700 PCR thermal cycler (Applied Biosystems, Forster City, CA, USA) using the following cycling conditions 95°C for 5min., 45 cycles of 95°C for 15s, 65°C for 15s, and 72°C for 10min. The products were detected on 1.5% agarose gel stained with ethidium bromide. Profiles were compared to those of *A. cantonensis* and *A. costaricensis* established by Caldeira *et al*. (2003).

Table 1. Prevalence of infection by *A. cantonensis* in *A. fulica* pooled samples by region in the municipality of São Bernardo do Campo (SP, Brazil) and the absolute number of parasitic loads per pool

Region	Number of *A. fulica snails* collected	Number of pools per region	Number of pools with nematode larvae	Number of nematode larvae per pool	Number of pools positive for *A. cantonensis*
Rudge Ramos	10	3	1	84	--
Baeta Neves	10	3	--	--	--
Planalto	14	3	2	27; 102	2
Demarchi	10	3	1	46	1
Dos Alvarengas	10	3	2	42; 68	2
Batistini	10	3	3	29; 31; 74	3
Montanhão	10	3	2	12; 47	2
Rio Grande	16	4	3	23; 76; 112	3
Total	90	25	14	773	13

Results show that, with the exception of the Baeta Neves region, all others presented snails infected with nematode larvae. Six regions (Planalto, Demarchi, Dos Alvarengas, Batistini, Montanhão, Rio Grande) presented positive samples for *A. cantonensis*. No positive sample for *A. costaricensis* was found in any region. Numbers of *A. fulica snails* collected for the study, of pooled samples from each region, of pools with nematode larvae, of nematode larvae per pool and of pools infected with *A. cantonensis* among those with nematode larvae are shown in Table 1. Considering the *A. fulica* pooled samples, 56% (14/25) of them contained nematode larvae and 52% (13/25) were positive for *A. cantonensis*. Relative to the 8 regions, 87.5% (7/8) of them contained nematode larvae and 75% (6/8) were positive for *A. cantonensis*. Several snails play roles as intermediate hosts for *A. cantonensis* and, among them, the giant African snail *A. fulica* is one of the most important due to its abundance and occupation in different ecosystems (Giannelli *et al*., 2016; Guerino *et al*., 2017).

The presence of *A. cantonensis* in Brazil was first suspected in 2006 at the V Congress of Infectology, at which a clinical case of eosinophilic meningoencephalitis in the State of Rio de Janeiro that resulted in death 10 days after the ingestion of *A. fulica* was reported (Carvalho *et al*., 2012). After the first report of *A.*

cantonensis in Brazil by Caldeira *et al*. (2007), in which it was observed that 66% of the collected *A. fulica* snails were infected with such parasite, several other investigations described its presence in *A. fulica* from harbor areas of this country (i.e., Ilhéus, Bahia; Angra dos Reis, Rio de Janeiro; Paranaguá, Paraná; Navegantes, Santa Catarina) (Carvalho *et al*., 2012). In the Baixada Santista region (São Paulo, Brazil), an investigation similar to our study was previously performed and 21.7% of the *A. fulica* snails analyzed were infected with *A. cantonensis* (Guerino *et al*., 2017).

The presence of *A. fulica* snails naturally infected with *A. cantonensis* should serve as a warning to public health authorities about the potential risk of infection to humans. Based on our results *A. costaricensis* did not seem to have much zoonotic importance when the intermediate host was *A. fulica*, since no positive sample was found, but it would be precipitous to say that this parasite does not infect such mollusk. By experimentally infecting *A. fulica* with *A. costaricensis* larvae, it was shown that *A. fulica* is, in fact, susceptible to the parasite, requiring, however, high levels of larval exposure. Although only 32% of the samples were found to be positive, the parasite burden in 42% of the snails ranged from 200 to 1000 third stage larvae (L3) (Carvalho *et al*., 2003).

These data are useful to public health authorities in order to establish policies related to surveillance and planning of preventive actions. For example, implementing simple wash protocols for vegetables, public education on the dangers of raw mollusk consumption and rat and mollusk control measures may be extremely helpful (Barrett *et al*., 2016). Considering the potentially lethal nature of angiostrongyliasis and its increasing geographical range, it is important that this disease is given proper consideration.

Keywords: Achatina fulica, Angiostrongylus cantonensis, Angiostrongylus costaricensis, *prevalence, city of São Bernardo do Campo*

RESUMO

Angiostrongylus cantonensis e Angiostrongylus costaricensis *são nematoides parasitas que residem em roedores e usam gastrópodes como hospedeiros intermediários*. Achatina fulica, *conhecida como caramujo-gigante-africano, é um hospedeiro intermediário que desempenha importante papel na dispersão de* A. cantonensis e A. costaricensis, *patógenos zoonóticos que causam, respectivamente, meningoencefalite eosinofílica e angiostrongilíase abdominal em humanos. O objetivo deste estudo foi o de coletar caramujos* (A. fulica, *n=90) de oito regiões diferentes (Rudge Ramos, Baeta Neves, Planalto, Demarchi, Dos Alvarengas, Batistini, Montanhão, Rio Grande) da cidade de São Bernardo do Campo (SP) para detecção molecular de* A. cantonensis *ou* A. costaricensis *pela técnica de reação em cadeia da polimerase (PCR). As amostras foram processadas em* pools *(n=25) segundo a região de coleta. Com exceção da região de Baeta Neves, as demais apresentaram caramujos infectados com larvas de nematoides. Seis regiões apresentaram amostras positivas para* A. cantonensis. *Quatorze (56%) das 25 amostras em* pool *apresentaram larvas de nematoides, e 52% delas (13/25) foram positivas para* A. cantonensis. *Nenhuma amostra positiva para* A. costaricensis *foi encontrada. A presença de* A. fulica *naturalmente infectada por* A. cantonensis *deve servir como um alerta para as autoridades de saúde pública sobre o risco potencial de infecção para humanos.*

Palavras-chave: Achatina fulica, Angiostrongylus cantonensis, Angiostrongylus costaricensis, *prevalência, São Bernardo do Campo*

REFERENCES

BARRATT, J.; CHAN, D.; SANDARADURA, I.; MALIK, R. *et al*. *Angiostrongylus cantonensis*: a review of its distribution, molecular biology and clinical significance as a human pathogen. *Parasitology*, v.143, p.1087-1118, 2016.

CALDEIRA, R.L.; CARVALHO, O.S.; MENDONÇA, C.L.F.G.; GRAEFF-TEIXEIRA, C. *et al*. Molecular differentiation of *Angiostrongylus costaricensis*, *Angiostrongylus cantonensis* and *A. vasorum* by polymerase chain reaction and restriction fragment length polymorphism. *Mem. Inst. Oswaldo Cruz*, v.98, p.1039-1043, 2003.

CALDEIRA, R.L.; MENDONÇA, C.L.G.F.; GOVEIA, C.O.; LENZI, H.L. *et al*. First record of molluscs naturally infected with *Angiostrongylus cantonensis* (Chen, 1935) (Nematoda: Metastrongylidae) in Brazil. *Mem. Inst. Oswaldo Cruz*, v.102, p.887-889, 2007.

CARVALHO, O.M.; TELES, H.M.S.; MOTA, E.M.; MENDONÇA, C.L.G.F.; LENZI, H.L. Potentiality of *Achatina fulica* Bowdich, 1822 (Mollusca: Gastropoda) as intermediate host of the *Angiostrongylus costaricensis* Morera & Céspedes 1971. *Rev.Soc. Bras. Med. Trop*., v.36, p.743-745, 2003.

CARVALHO, O.S.; SCHOLTE, R.G.C.; MENDONÇA, C.L.F.; PASSOS, L.K.J.; CALDEIRA, R.L. Angiostrongylus cantonensis (Nematoda, Metastrongyloidea) in molluscs from harbor areas in Brazil. *Mem. Inst. Oswaldo Cruz*, v.107, p.740-746, 2012.

ESPÍRITO-SANTO, M.C.; PINTO, P.L.S.; MOTA, D.J.G.; GRYSCHEK, R.C.B. The first case of *Angiostrongylus cantonensis e*osinophilic meningitis diagnosed in the city of São Paulo, Brazil. *Rev. Inst. Med. Trop*., v.55, p.129-132, 2013.

GASSER, R.B.; CHILTON, N.B.; HOSTE, H.; BEVERIDGE, I. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Res.*, v.21, p.2525-2526, 1993.

GIANNELLI, A.; CANTACESSI, C.; COLELLA, V.; DANTAS-TORRES, F.; OTRANTO, D. Gastropod-borne helminths: a look at the snail-parasite interplay. *Trends Parasitol.*, v.32, p.255-264, 2016.

GRAEEF-TEIXEIRA, C. Expansion of *Achatina fulica* in Brazil and potential increased risk for angiostrongyliasis. *Trans. Rev. Soc. Trop. Med. Hyg*., v.10, p.743-744, 2007.

GUERINO, L.R.; PECORA, I.L.; MIRANDA, M.S.; AGUIAR-SILVA, C. *et al*. Prevalence and distribution of *Angiostrongylus cantonensis* (Nematoda, Angiostrongylidae) in *Achatina fulica* (Mollusca, Gastropoda) in Baixada Santista, São Paulo, Brazil. *Rev. Soc. Bras. Med. Trop*., v.50, p.92-98, 2017.

KLIKST, M.M.; PALUMBO, N.E. Eosinophilic meningitis beyond the Pacific basin: the global dispersal of a peridomestic zoonosis caused by *Angiostrongylus cantonensis*, the nematode lungworm of rats. *Soc. Sci. Med*, v.34, p.199-212, 1992.

MORASSUTTI, A.L.; TIENGO, S.C.; FERNANDEZ, M.; ASAWANYAWISUTH, K.; GRAEFF-TEIXEIRA, C. Eoshinophilic meningitis caused by *Angiostrongylus cantonensis*: an emergent disease in Brazil. *Mem. Inst. Oswaldo Cruz*, v.109, p.399-407, 2014.

PIEN, F.D.; PIEN, B.C. *Angiostrongylus cantonensis* eosinophilic meningitis. *Int. J. Infect. Dis*., v.3, p.161-163, 1999.

SILVA, A.C.A; GRA'EFF-TEIXEIRA, C.; ZAHA, A. Diagnosis of abdominal angiotrongyliasis by PCR from sera of patients. Rev. Inst. Med. Trop. S. Paulo, v.45, p.295-297, 2003.