In vitro maintenance of *Angiostrongylus costaricensis* does not provide physiological conditions for egg laying

Manutenção *in vitro* de *Angiostrongylus costaricensis* não constitui condição fisiológica para ovipostura

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ABSTRACT

Fifteen pairs (male/female) of Angiostrongylus costaricensis *were kept* in vitro *in Waymouth medium for three days to evaluate the amount and duration of egg laying. At 24, 48 and 72 hours, the mean egg counts were 321, 24 and 4 eggs/ 10 microliters, respectively. Most of the eggs were eliminated within the first 24 hours, suggesting they are expelled under non-physiological conditions. These results indicate that* in vitro *conditions are not appropriate for drug trials of egg-laying inhibitors for treatment of abdominal angiostrongylosis.*

Key-words: Angiostrongylus costaricensis. *Abdominal angiostrongyliasis. Egg laying.*

RESUMO

Quinze duplas (macho/fêmea) de Angiostrongylus costaricensis *foram mantidas* in vitro*, em meio de Waymouth durante 3 dias, para observação da quantidade e duração da oviposição. Médias de 321, 24 e 4 ovos em 10 microlitros foram registradas em 24, 48 e 72 horas, respectivamente. A maioria dos ovos foi eliminada nas primeiras 24 horas, sugerindo terem sido expulsos em condições não fisiológicas. Estes resultados indicam que as condições* in vitro *não são adequadas para testes de drogas inibidoras da oviposição, para tratamento da angiostrongilíase abdominal.*

Palavras-chaves: Angiostrongylus costaricensis. *Angiostrongilíase abdominal. Oviposição.*

Angiostrongylus costaricensis Morera & Cespedes, 1971, is a parasite nematode of the mesenteric arterial system in wild rodents[5 16 19]. The intermediate hosts are terrestrial mollusks, mostly of the Veronicellidae family[8 18]. Abdominal angiostrongylosis may result from accidental human infection, leading to severe ischemic and inflammatory intestinal lesions[4 6 17]. Since the first report in Costa Rica[19], many human cases have been reported from Mexico[14 15] to Brazil[1 2 3 6 13 23 24]. Besides its medical importance, *Angiostrongylus costaricensis* has been selected as a model to study the diseases caused by tissue nematodes and the efficacy of anthelmintics[20]. The large scale production of larvae and worms, as well as the principle of minimizing laboratory animal use justifies the need for *in vitro* maintenance of parasites. None of the reports from trials of *in vitro* cultivation of *A. costaricensis* were concerned with the quantity and duration of egg laying[10 11 12 20 21]. However, these data are important to evaluate the usefulness of an *in vitro* model for drug therapy of abdominal angiostrongylosis. Eggs are considered one of the main causative factors for granulomatous inflammatory reaction in human disease. The objectives of this experiment were to evaluate the quantity and duration of *in vitro* egg laying by *Angiostrongylus costaricensis*. Male, 6-week-old, *Mus domesticus*, Swiss strain, were used as the definitive host and were inoculated (*per os*) with 10 infective third-stage larvae of *A. costaricensis* (Santa Rosa strain) each. These were obtained from experimentally-infected *Biomphalaria glabrata* snails, by artificial digestion using 0.03% pepsin (Sigma P 7125) solution in 0.7% HCL for 2 hours at 37ºC[8 22]. Following exposure to larvae, each group of five animals were caged and given food and water *ad libitum*. All surviving mice were sacrificed at 34 days post infection (dpi) through anesthetic depression. Adult worms were recovered from the mesenteric artery, transferred into Petri dishes and washed with PBS.

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After washing, a total of 15 male/female pairs were separated and transferred onto another plate, containing Waymouth medium (GIBCO) plus antibiotic and amphotericin and incubated at 37ºC and 5% CO^2. The medium was changed at 1-day intervals. Eggs were examined under a stereomicroscope and counted, every 24 hours, for 72 hours. Most of the eggs were recovered in the first 24 hours, with a mean 321 eggs/female/10mL. After 48 hours, the number of eggs decreased dramatically, to 24 eggs/female/10mL. After 72 hours, only a few dark and deformed eggs were recovered (Table 1).

Table 1 - Daily recovery of Angiostrongylus costaricensis ***eggs per mL from 15 pairs (male/female) of parasites maintained in Waymouth medium for 72 hours.***

Pairs	Number of eggs/10μL		
	24h	48h	72h
1	420	70	8**
2	510	9	0
3	142	100	2**
4	460	30*	0
5	270	4	0
6	467	10	0
7	260	2	0
8	320	26	0
9	230	5	0
10	250	81	0
11	300	14	0
12	270	0	0
13	450	0	0
14	230	24**	8**
15	230	34	50**

* anembryonated ** dark and deformed

Chemically defined Waymouth medium, is reportedly the best culture medium for the development of L3 into young adult stage, with a 60% success rate[10 11 12]. Otherwise, when mature female worms removed from the definitive host are kept in the same culture medium, a very intense and extensive egg elimination takes place, suggestive of a stress response.

In conclusion, these data indicate that the *in vitro* conditions tested are not a good experimental model for testing drug therapy for abdominal angiostrongylosis.

REFERENCES

1. Agostini AA, Marcolan AM, Lisot JMC, Lisot JUF. Angiostrongilíase abdominal, estudo anátomo-patológico de quatro casos observados no Rio Grande do Sul, Brasil. Memórias do Instituto Oswaldo Cruz 79: 443-445, 1984.
2. Ayala MAR. Angiostrongiloidíase abdominal: seis casos observados no Paraná e em Santa Catarina, Brasil. Memórias do Instituto Oswaldo Cruz 82: 29-36, 1987.
3. Ayala MAR, Guerra IF, Schir RA, Motizuki A .Angiostrongiloidose abdominal. Apresentação de um caso. Memórias do Instituto Oswaldo Cruz 77: 189-193, 1982.
4. Céspedes R, Salas J, Mekbe S, Troper L, Müllner F, Morera P. Granulomas entéricos e linfáticos com intensa eosinofilia tisular producidos por un strongilídeo (strongylata). Acta Médica Costaricensis 10:235-255, 1967.
5. Graeff-Teixeira C, Avila-Pires FD, Machado, RCC, Camillo-Coura L, Lenzi HL. Identificação de roedores silvestres como hospedeiros do *Angiostrongylus costaricensis* no Sul do Brasil. Revista do Instituto de Medicina Tropical de São Paulo 32: 147-150, 1990.
6. Graeff-Teixeira C, Camillo-Coura L, Lenzi Hl. Clinical and epidemiological aspects of abdominal angiostrongyliasis in Southern Brazil. Revista do Instituto de Medicina Tropical de São Paulo 33: 373-378, 1991.
7. Graeff-Teixeira C. Camillo-Coura L., Lenzi Hl. Histopathological criteria for the diagnosis of abdominal angiostrongyliasis. Parasitology Research 77: 606-611, 1991b.
8. Graeff-Teixeira C, Morera P. Método de digestão de moluscos em ácido clorídrico para isolamento de larvas de metastrongilídeo. Biociências 3:85-89, 1995.
9. Graeff-Teixeira C, Thomé JW, Pinto SCC, Camillo-Coura L, Lenzi HL. *Phyllocaulis variegatus* an intermediate host of *Angiostrongylus costaricensis* in South Brazil. Memórias do Instituto Oswaldo Cruz 84:65-68, 1989.
10. Hata H. Essential amino acids and other essential components for development of *Angiostrongylus costaricensis* from third-stage larvae to young adults. Journal of Parasitology 80: 518-520, 1994.
11. Hata H. *In vitro* cultivation of *Angiostrongylus costaricensis* eggs to first stage larvae in chemically defined medium. International Journal for Parasitology 26: 281-286, 1996.
12. Hata H, Kojima S. *Angiostrongylus costaricensis*: Culture of third-stage larvae to young adults in a Defined Medium. Experimental Parasitology 73: 354-361, 1991.
13. Iabuki K, Montenegro MR. Apendicite por *Angiostrongylus costaricensis*. Apresentação de um caso. Revista do Instituto de Medicina Tropical de São Paulo 21: 33-36, 1979.
14. Lobo-Sanahuja F, Loria-Cortes R, Gonzalez G. Angiostrongilosis abdominal. Aspectos clínicos, tratamento y revisión de literatura. Boletin Médico Hospital Infantil 44: 4-9, 1987.
15. Loria-Cortes R, Lobo Sanahuja JF. Clinical abdominal angiostrongylosis. A study of 116 children with intestinal eosinophilic granuloma caused by *Angiostrongylus costaricensis*. American Journal of Tropical Medicine and Hygiene 29: 538-544, 1980.
16. Morera P. Investigación del huésped definitivo de *Angiostrongylus costaricensis* (Morera y Céspedes, 1971). Boletin Chileno de Parasitologia 25: 133-134, 1970.
17. Morera P. Abdominal angiostrongyliasis: a problem of public health. Parasitology Today 1: 173-175, 1985.
18. Morera P, Ash LR. Investigación del huésped intermediário de *Angiostrongylus costaricensis* (Morera & Cespedes, 1971). Boletin Chileno de Parasitologia 25:135, 1970.
19. Morera P, Céspedes R. Angiostrongylose abdominal. Una nueva parasitosis humana. Acta Médica Costa Rica 14: 159-173, 1971.
20. Terada M, Rodriguez O, Dharejo AM, Ishii AI , Sano M. Studies on chemotherapy of parasitic helminths. (XXVI) Comparative in vitro effects of various anthelmintics on the motility of *Angiostrongylus costaricensis* and *Angiostrongylus cantonensis*. Japanese Journal of Parasitology 35: 365-367, 1986.
21. Uga S, Araki K, Matsumura T. Deposition and development of eggs of *ngiostrongylus cantonensis in vitro*. Japanese Journal of Parasitology 3: 257-264, 1984.
22. Wallace GD, Rosen L. Techniques for recovering and identifying larvae of angiostrongylus cantonensis from molluscs. Malacologia 7: 427-438, 1969.
23. Zambrano PZ. Ileocolitis pseudotumoral eosinofilica de origem parasitário. Revista Latinoamericana de Patologia 12: 43-50, 1973.
24. Ziliotto A, Kunzle JE, Fernandes LAR, Prates-Campos JC, Britto-Costa R. Angiostrongilíase: apresentação de um provável caso. Revista do Instituto de Medicina Tropical de São Paulo 17: 312-318, 1975.