NOTA

A DOUBLE GAUZE FILTER MODIFICATION OF BAERMANN METHOD FOR ISOLATION OF *Angiostrongylus costaricensis* LARVAE FROM RODENT FECES

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ABSTRACT

The Baermann method is a classical method for isolation of nematode larvae from either biological material or soil. The inclusion of a second gauze filter in the funnel resulted in a cleaner suspension of *Angiostrongylus costaricensis* first stage larvae (L1) from experimentally infected rodent feces, without retention of L1 as compared to the original method, if the preparation is left at rest for 12 hours. The second filter is a 2x2 cm gauze (4 layers) inserted in the opening of the straight portion of the funnel. A cleaner larvae suspension is important as a first step for purification and decontamination of the parasites to be cultivated *in vitro*.

KEYWORDS: *Angiostrongylus costaricensis*. Baermann method. Larvae isolation

Angiostrongylus costaricensis Morera and Céspedes, 1971 is a nematode parasitic of wild rodents in the Americas (Morera 1973). The worms live inside portal-mesenteric blood vessels and the first stage (L1) larvae are eliminated in feces. Accidental human infection may produce severe abdominal disease (Graeff-Teixeira et al. 1991). Improvement of molecular diagnostic tests is required for better management of suspected infections in endemic areas. It is also important for non-endemic areas, since several imported cases have been documented in Europe and United States (Silveira et al. 1989, Vázquez et al. 1993). The development both of nucleic acid detection tests and immunological tests, requires the production of parasite stages in large amounts. The *in vitro* cultivation of *A. costaricensis* is possible with the exception of the evolution from L1 to third stage larvae (L3) – the infective form for vertebrates (Hata & Kojima 1991). Trials with several media have been unsuccessful and one frequent

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problem is bacterial and fungal contamination. Isolation with a Percoll gradient greatly improves isolation of L1 from rodent feces (Graeff-Teixeira et al. 1999), but contamination still prevents the long term *in vitro* maintenance of larvae. In order to reduce the amount of debris co-isolated with the L1 through the Baermann method, a modification was introduced: a four layer gauze filter was inserted in the straight portion of the glass funnel.

Oligoryomis nigripes is a natural host for *A. costaricensis* in southern Brazil (Graeff-Teixeira et al 1990) and it has been infected experimentally for L1 production in the laboratory. 0,2 g of uninfected rodent fecal pellets were placed on surgical gauze (4 layers) and an aqueous suspension of 300 L1 was slowly dropped over the pellets. Baermann funnels were filled with tap water at room temperature and 4 layers of gauze (2x2 cm) was inserted in the straight portion of the funnels (DF= double filter). The gauze with the feces, sustained by a plastic mesh was placed in the large opening of the funnel (diameter = 10 cm). After 1, 2 and 12 hours (overnight), approximately 0.5 ml was removed and examined under a stereomicroscope for quantification of L1. As a control, the same procedures were done with funnels where the lower gauze filter was lacking (SF = single filter). The average cumulative number of L1 recovered at each time point is presented in the Figure 1.

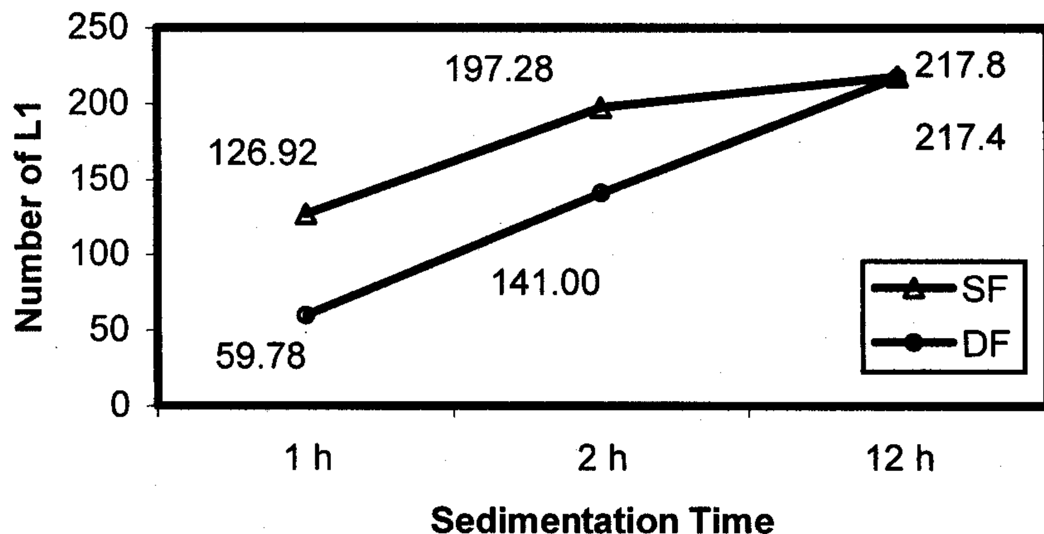

Figure 1. Cumulative number of L1 obtained after 1, 2 and 12 hours of sedimentation in funnels with one (SF) or two (DF) sets of filter gauze. Some retention of L1 occurs with sedimentation for 1 hour (t=4.3265; T-test, significant) and 2 hours, but there is no difference after 12 hours (t=0.0131; not-significant).

The number of larvae recovered after 1 hour was significantly lower with DF (T-test; t=4.3265; significant when t > 2.145). With 2 hours there was also a lower but not-significant L1 number (t= 2.0167). Approximately 72.6% and 72.5%

of the 300 L1 were recovered by the two methods after 12 hours (t= 0.0131). On a subjective evaluation, a cleaner L1 suspension was obtained with the DF method.

In conclusion, the modification of Baermann method with insertion of a second gauze filter in the straight portion of the funnel produces a cleaner larvae suspension, without larvae retention at the sedimentation time of 12 hours as compared to the original method.

RESUMO

Duplo filtro de gaze: modificação do método de Baermann para isolamento de larvas de *Angiostrongylus costaricensis* das fezes de roedores.

O método de Baermann é considerado clássico para o isolamento de larvas de nematódeos tanto a partir de material biológico quanto do solo. A inclusão de um segundo filtro de gaze no funil resultou em uma suspensão mais limpa de larvas de primeiro estágio (L1) de *Angiostrongylus costaricensis* a partir de fezes de roedores experimentalmente infectados, sem retenção de L1, quando comparado com o método original com um tempo de sedimentação de 12 horas. O segundo filtro constituiu-se de quatro camadas de gaze, medindo 2x2 cm, inseridas na abertura da porção reta do funil. Suspensões larvares mais limpas são importantes como primeiro passo na purificação e descontaminação dos parasitas a serem cultivados *in vitro*.

DESCRITORES: *Angiostrongylus costaricensis*. Método de Baermann. Isolamento de larvas.

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