
**EVIDENCES FOR CLUSTERING OF *Schistosoma mansoni*
EGGS IN THE SEDIMENT PRODUCED BY HELMINTEX**

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ABSTRACT

Diagnosis of schistosomiasis in low intensity transmission areas requires the improvement of methods in order to overcome the lack of sensitivity of classical coproparasitological methods. Helmintex is a new method based on the interaction of *Schistosoma mansoni* eggs with paramagnetic beads in a magnetic field. A preliminary seeding experiment gave support to a previous impression from routine examinations with Helmintex that eggs are usually found in the bottom half rather than in the upper half of the column of the sediment under examination. Nine replicas of one hundred eggs were seeded in uninfected 30g feces and each replica was submitted to Helmintex. The top 40 µL aliquots of sediment were removed and sequentially examined under the microscope for enumeration of eggs. In 6 samples most of the eggs were found at the bottom half. This interesting finding may lead to an improved final step on egg isolation and better sensitivity for coproscopic diagnosis of schistosomiasis.

KEY WORDS: *Schistosoma mansoni*. Coproparasitological diagnosis. Schistosomiasis. Low endemicity.

Several new methods with improved sensitivity are under evaluation for diagnosis of schistosomiasis in areas with low intensity of transmission where low egg burden in feces is a rule (Corachan 2002; Graeff-Teixeira et al., 2004; Juberg et al., 2008; Coelho et al, 2009). Helmintex is based on the interaction of eggs with paramagnetic beads and it has an estimated sensitivity of 100% at the lower detection limit of 1.3 egg per gram of feces (Teixeira et al., 2007). Investigations on the mechanism underlying the mobilization of *S. mansoni* eggs when they are submitted to a magnetic field and detailed evaluation of recovery rates in seeding

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experiments are underway. This short note aims to report evidence for clustering of *Schistosoma mansoni* eggs amid the final sediment resulting from the sequential steps of concentration and magnetic isolation.

The examination of stool samples from individuals at risk for the infection at the low intensity transmission focus of schistosomiasis in Esteio, Rio Grande do Sul, Brazil produced the preliminary indications that eggs are usually found in the last amounts of sediment under study at the microscope.

As part of an extensive determination of recovery of eggs through Helminthex method, 100 eggs were obtained from digestion of livers from experimentally infected mice and seeded into 10 replicas of uninfected 30g fecal samples.

Helminthex was performed as previously described (Teixeira et al., 2007). Briefly, feces were filtered through 4 layers of surgical gauze, washed by spontaneous sedimentation until a clear supernatant was produced, sieved through 100, 200 and 325 mesh per square inches, centrifuged after homogenization with ethyl-acetate (Ritchie), incubated for 1h at room temperature with a suspension of paramagnetic beads (Bangs Labs, EUA) and, finally, the micro tubes containing the sediment were exposed to the magnet (Dynabeads) for 10 minutes, and the supernatant was discarded. The final sediment was totally examined at optical microscopy, magnification x100. For each slide, the top 40 µL of the sediment was removed and mixed with 40 µL of a 10% formalin solution. The sequential ordering of slides represents the examination from top to bottom of the column of sediment inside a 1.5 mL micro-tube.

One replica was discarded after accidental leaking and partial loss of the sediment. The examination of 9 sediments resulted in recovery rates ranging from 1 to 10 eggs, what is lower than the rates found by Teixeira et al (2007) (Table 1). Most of the eggs were found in the bottom half of the sediment in 6 out of 9 samples (Figure 1).

Table 1. Results from a seeding experiment with 100 *Schistosoma mansoni* eggs in 30 g of uninfected feces, concentration through Helminthex method and detection at the microscope

Replicas	1	2	3	4	5	6	7	8	9
Percentage of eggs found in each column of sediment									
Upper	13	25	20	60	60	50	0	0	0
Lower	87	75	80	40	40	50	100	100	100
Number of slides	23	25	31	30	13	12	6	11	9
Total Eggs	8	4	5	10	5	4	1	2	2
Egg	0,3	13,3	16,7	33,3	16,7	13,3	3,3	6,7	6,7

Examination of a large amount of feces is essential for increased sensitivity of stools examinations, but it also results in a labor intensive method, like Helminthex.

Eggs settling down in the column of sediment may represent an opportunity to improve sensitivity and to a less labor intensive Helminex method through application of the final sediment into a density gradient like Percoll (Eberl et al., 2002). Additionally, these data may also lead to priority examination of the bottom half of the sediment. Very sensitive diagnostic methods like Helminex are essential for optimization of control measures of schistosomiasis in low intensity transmission areas.

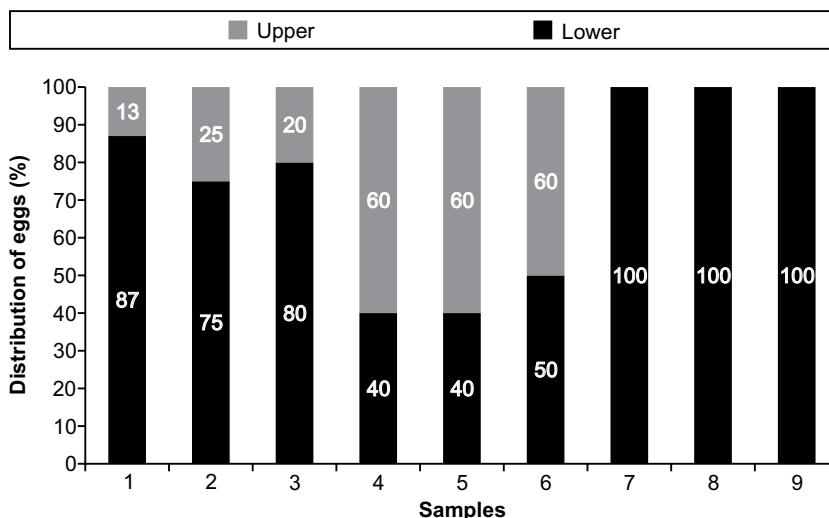


Figure 1. Histogram showing the percentage recovery of *Schistosoma mansoni* eggs in 9 replicas of a seeding experiment with 100 eggs in 30 g of uninfected feces, concentrated through Helminex and with examination at the microscope of the upper and lower half of the final sediment.

RESUMO

Evidências para a aglomeração de ovos no sedimento produzido pelo Helminex

O diagnóstico da esquistossomose em áreas de baixa intensidade de transmissão exige o aprimoramento dos métodos para superar a pouca sensibilidade dos métodos coproparasitológicos clássicos. Helminex é um método novo, baseado na interação dos ovos de *Schistosoma mansoni* com partículas paramagnéticas em um campo magnético. Um estudo preliminar de semeadura confirmou a impressão obtida durante exames de rotina com Helminex, de que os ovos são geralmente encontrados na metade inferior e não na metade superior da coluna de sedimento sob exame. Nove réplicas de 100 ovos foram semeados em fezes não infectadas e cada réplica foi submetida ao Helminex. Alíquotas de 40 μ L foram retiradas do

topo e sequencialmente examinadas ao microscópio óptico, para contagem dos ovos. Em 6 replicas, a maioria dos ovos foram encontrados na metade inferior. Estes achados interessantes podem levar ao aprimoramento da etapa final de isolamento dos ovos e maior sensibilidade no diagnóstico coproscópico da esquistossomose.

DESCRITORES: *Schistosoma mansoni*. Diagnóstico coproscópico. Esquistossomose.

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