Morbidity due to *Schistosoma mansoni* - *Entamoeba histolytica* coinfection in hamsters (*Mesocricetus auratus*)

**Morbidade em hamsters (*Mesocricetus auratus*) devido à co-infecção *Schistosoma mansoni* - *Entamoeba histolytica***

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**ABSTRACT**

Data on *Schistosoma mansoni*-Entamoeba histolytica coinfection are scarce in the literature. In the present study, hamsters that had been infected for 70 days with *Schistosoma mansoni* (LE strain) were inoculated via the portal vein with two strains of trophozoites of Entamoeba histolytica: ICB-EGG (highly virulent) and ICB-RPS (non-virulent). The most evident result of coinfection was increased morbidity and mortality, in comparison with either of the infections alone. Histologically, there were no evident signs of interaction between these two infections. The morphological findings of schistosomal granuloma and amoebic abscesses in the liver were similar to those seen in the respective single-infection controls. However, there was severe wasting of the animals with both infections and greater numbers of amoebic lesions in their livers. The results obtained indicated that schistosomiasis aggravates the course of amoebiasis in hamsters.


**Resumo**

Dados sobre a co-infecção *Schistosoma mansoni*-Entamoeba histolytica são escassos na literatura. No presente estudo, hamsters com 70 dias de infecção por *Schistosoma mansoni* (cepa LE) foram inoculados com trofozoítos de *Entamoeba histolytica*, cepa ICB-EGG (virulenta) e cepa ICB-RPS (não virulenta), via veia porta. O mais evidente resultado da co-infecção foi o aumento da morbidade e mortalidade, quando comparado com os animais com somente uma das infecções. Histologicamente, não houve sinais evidentes da interação entre as duas infecções. O aspecto morfológico do granuloma esquistossomótico e do abcesso hepático amebiano são similares aos observados nos controles, com somente uma infecção. Entretanto, foi observado que os animais co-infetados apresentavam-se mais debilitados e com maior número de lesões amebianas no fígado. Os resultados obtidos indicam que a esquistossomose agrava o curso da infecção amebiana em hamsters.


Schistosomiasis caused by the presence of *Schistosoma mansoni* in the visceral system of its hosts affects about 130 million people worldwide. The egg-laying in the tissues, mainly in the liver, leads to a granulomatous inflammatory process that changes the hepatic microenvironment (hypertension and periporal fibrosis). This may, in some patients, result in severe or hepatosplenic forms of the disease, which is potentially lethal because of the bleeding caused by disruption of the esophageal varices.

Amoebiasis is a disease caused by the protozoan *Entamoeba histolytica*, with estimated worldwide prevalence of 500 million infected people¹². The natural habitat of the parasite is the large intestine, and many cases are asymptomatic. Nevertheless, several factors (parasite strain, bacterial flora or host immunological condition) allow the protozoan to invade the intestinal mucosa, and possibly to reach a site distant from its usual habitat, thus resulting in extra-intestinal amoebiasis. The liver is commonly the extra-intestinal organ most affected⁶ ⁹ ²⁰.
The prevalence of *E. histolytica* infection in the human population is high in most of the different regions of the world where schistosomiasis is also an endemic disease, with consequently increased morbidity and mortality. By reviewing the literature, it can be seen that few studies have dealt with *S. mansoni*/*E. histolytica* interaction. To our knowledge, there are no experimental studies using intrahepatic inoculation for coinfection of hamsters, although the liver is recognized as the main site for the lesions resulting from granulomas caused by *S. mansoni* eggs, as well as being the most common site for extra-intestinal amoebiasis.

Multiparasitism is a frequent event in human populations living in areas that are endemic for schistosomiasis, and it mainly affects disadvantaged populations. Thus, studying schistosomiasis in association with other pathogens may be pivotal in updating with new approaches on this subject. Several studies have indicated that there are endemic areas for schistosomiasis and other areas for amoebiasis, and also many regions where both diseases coexist.

In the present experimental study, the interaction between *S. mansoni* and *E. histolytica* was investigated. Animals previously infected with *S. mansoni* (ICB-EGG) were then inoculated with trophozoites of *E. histolytica* (ICB-EGG and ICB-RPS strains) via the portal vein and intracecal inoculation. The ICB-EGG strain presented a high degree of virulence, whereas the ICB-RPS strain was used as an infection control, since it was not virulent for the animals used in this experiment. Animals bearing coinfection (*S. mansoni* - LE strain and *E. histolytica* - ICB-EGG strain) exhibited higher morbidity and mortality than did single-infected controls, although no special features were evident in the histological patterns of the hepatic lesions due either to amoebiasis or to schistosomiasis.

**MATERIAL AND METHODS**

**Entamoeba histolytica** strains. ICB-EGG strain. This was isolated in May 1988, by Dr. Silva, from mucosanguinolent feces of a symptomatic male patient, coming from Manaus (Amazonas, Brazil), containing cysts and trophozoites. The patient presented dysenteric colitis and hepatic abscess, with positive serology for HAI, ELISA and IIF. Inoculation of this strain into experimental animals resulted in 100% infection in hamsters (grades III and IV), and 63% intracecal infection in rats (grades II and III), when an inoculum of 2.5 x 10⁵ trophozoites was used. To our knowledge, there are no experimental studies using intrahepatic inoculation for coinfection of hamsters, although the liver is recognized as the main site for the lesions resulting from granulomas caused by *S. mansoni* eggs, as well as being the most common site for extra-intestinal amoebiasis.

Experimental animals. Four to six-week-old hamsters (*Mesocricetus auratus*) of both sexes, weighing approximately 100g, were provided by the animal house of the Institute of Biological Sciences/UFMG and kept in separated cages (8-14 animals). They were given a standard balanced allowance of food every day, and water *ad libitum*. For intracecal inoculations, Wistar rats of both sexes and weighing approximately 200g were used.

Parasites and experimental infection. In order to produce *S. mansoni* infection, the animals were subcutaneously inoculated with approximately 25 cercaria/animal (LE strain). After 70 days of cercarial infection, the animals were inoculated intraperitoneally with trophozoites, in accordance with the procedure described by Rocha and Coelho. Shortly afterwards, under asepsis and anesthesia, the abdominal wall of the animals was opened by means of a horizontal incision of 1.5cm length. Using a sterilized hypodermic syringe, the trophozoites were inoculated into the portal vein and soon afterwards, the vein was compressed with a gauze embedded in 0.9% saline, to control the hemorrhage. Then, the peritoneum and the skin were individually sutured with nonabsorbent threads. The trophozoites were obtained from monoxenic cultures with *Crithidia fasciculata* at the log phase of growth (48-72 hours). The inoculum was calculated to obtain 2.5 x 10⁴ trophozoites in a total volume of 200µl.

In order to perform intracecal inoculations, the rats were kept without solid food for a 24h-period. After anesthesia and asepsis of the abdomen, the cecum was observed via an incision in the lower left quadrant. The exposed cecum was inoculated with 2.5 x 10⁴ trophozoites of *E. histolytica* (EGG strain), in 200µl volume. Inoculations were carried out into the normal or traumatized cecum, and the peritoneum and skin were sutured using nonabsorbent thread.

The animals subjected to surgery received analgesic (Banamine®/fluxinin - 2mg/kg) subcutaneously every 24h, for the first 48h following the surgery.

All the procedures relating to the EGG strain were conducted using a non-pathogenic RPS strain.

Animal sacrifice and evaluation of infection. One week after infection with *E. histolytica*, the animals were sacrificed by cervical fracture and subjected to necropsy. After opening the abdomen, the gross changes in the liver were evaluated. The proportion of animals with lesions containing amoebae per number of inoculated animals and the severity of the lesions were determined. The hepatic lesions were classified as reported by Diamond et al: grade 0 – liver without visible lesion or just whitish points at the inoculation site; grade 1 – single abscess at...
In the group of animals infected with *S. mansoni* only, all hamsters exhibited numerous granulomas within the hepatic parenchyma and there was no mortality.

Hamsters inoculated with trophozoites from the RPS strain (non-pathogenic) did not show amoebic hepatic lesions when inoculated with amoebae only or in association with *S. mansoni*.

**Intra-cecal inoculation.** When the intracecal route was used for performing inoculation of trophozoites, no amoebic lesions could be seen in the coinfectected group. The cecal mucosa was thick, due to the presence of *S. mansoni* eggs. However, the content was altered and more diarrheic in the group coinfectected with the ICB-EGG strain, and six animals presented trophozoites of *E. bistolytica* when observed under the microscope.

**Histopathology.** The lesions produced by schistosomal eggs were characterized by granulomas, formed by a small number of macrophages around viable eggs and surrounded by a cuff of lymphocytes and mild fibrosis (Figure 2A). These lesions were totally independent from necrotic areas associated with amoebic infection. The lesions tended to be more focal in the hamsters with *S. mansoni* coinfection, and were characterized by areas of coagulative necrosis, involving lobular parenchyma, probably reflecting earlier lesions. Trophozoites were easily identified at the periphery of necrotic areas and sometimes within sinusoids in the vicinity. Around these necrotic areas, mild non-specific chronic inflammatory infiltrate composed mainly of mononuclear cells was observed. In animals without *S. mansoni* infection, the necrotic areas were more extensive and lytic. At the periphery of these necrotic areas, where numerous trophozoites were detected, the necrotic material stained more basophilic due to nuclear condensation and cellular debris. Surrounding these necrotic areas, non-specific moderately chronic inflammatory infiltrate composed mainly of mononuclear cells including lymphocytes and plasma cells was also observed, accompanied by a mild degree of fibrosis (Figure 2B).

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**RESULTS**

**Inoculation by portal vein route.** In the group of animals infected with *E. bistolytica* only, of ICB-EGG strain (Table 1 and Figure 1), 16 animals presented lesions of degrees III and IV one week after sacrifice. It was also observed that one animal showed only one small focus of amoebic infection, and four animals presented no lesions.

Among the animals with both infections, one of them was sacrificed before the planned time, because it presented cachexia with frequent diarrhea. At necropsy, this animal’s liver presented multiple foci of amoebic appearance. The microscopy on direct smears and cultures for trophozoites was positive. Among the 23 remaining animals, 11 died between 4 and 5 days after amoebic infection, presenting diffuse lesions (grades II and III degrees) in the liver. The rest of animals showed dissemination of small, well marked amoebic lesions (grades II and III), diffusely throughout the liver.

In the group of animals infected with *S. mansoni* only, all hamsters exhibited numerous granulomas within the hepatic parenchyma and there was no mortality.

Hamsters inoculated with trophozoites from the RPS strain (non-pathogenic) did not show amoebic hepatic lesions when inoculated with amoebae only or in association with *S. mansoni*.

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**Histopathology.** Representative fragments of the liver were fixed in buffered formalin 10%. They were then dehydrated, clarified, embedded in paraffin, sliced into thin sections of 3-5µm and stained with hematoxylin-eosin for conventional optical microscopy.

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### Table 1 - Results from experimental inoculations of trophozoites (ICB-EGG and ICB-RPS strains), into the portal vein of hamsters (with and without Schistosoma mansoni infection). The animals were sacrificed seven days after receiving the amoebic inoculum.

<table>
<thead>
<tr>
<th>Group</th>
<th>Infection</th>
<th>Mortality</th>
<th>% Lesion</th>
<th>Infection grade*</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>%</td>
<td>0</td>
<td>I</td>
</tr>
<tr>
<td>1</td>
<td>Eh-EGG</td>
<td>0/21</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Sm + Eh-EGG</td>
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<td>50.0</td>
<td>24</td>
</tr>
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<td>3</td>
<td>Eh-RPS</td>
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<td>0</td>
</tr>
<tr>
<td>4</td>
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<td>Sm</td>
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</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>0/10</td>
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*Infection grade, in accordance with Diamond et al; Sm: Schistosoma mansoni; Eh-EGG: Entamoeba bistolytica (EGG strain); Eh-RPS: Entamoeba bistolytica (RPS strain); - No: sign of lesion
DISCUSSION

As emphasized earlier, the literature provides only scarce information regarding the S. mansoni - E. histolytica association, and most of the data does not relate to studies using experimental models\textsuperscript{4 12 15}. Moreover, the conclusions resulted from epidemiological surveys carried out in communities with precarious sanitary conditions, which are a predisposing factor for the seeding of other parasitic infections. Thus, based on this latter observation, it cannot be concluded that the presence of schistosomiasis would be per se a facilitating factor for E. histolytica infection, since such individuals are routinely exposed to a great number of other parasites.

In this study, we utilized the E. histolytica strains ICB-EGG (well known to be virulent) and ICB-RPS (non-virulent). Both of these are kept in the Amoebiasis Laboratory, and the LE strain of S. mansoni is kept in the Schistosomiasis Research Unit Laboratory. These laboratories belong to the Parasitology Department, Institute of Biological Sciences, Federal University of Minas Gerais, Brazil. These strains had been standardized with regard to pathogenicity and virulence by using experimental animals. The hamster is the most commonly used animal model in studies on experimental hepatic amoebiasis produced by E. histolytica\textsuperscript{13}. The portal vein has been used as the infection route for E. histolytica\textsuperscript{16}, since this is the natural route used by trophozoites to reach the liver. We observed that the presence of granulomatous schistosomiasis lesions made the amoebic infection a serious risk factor for death among the coparasitized animals. The presence of both infections led to the death of 50% of the animals, and it seemed that the lesions of the two parasitoses were cumulative, although there was no association between the presence of granulomas and trophozoites. Among the factors that could be involved in this increased mortality, it may be suggested that the animal’s fragility was due to the effects of schistosomal infection. Regardless of the fact that the amoebic abscesses were smaller in the animals with both infections, these abscesses were diffuse in the liver, thus resulting in numerous foci of infection, with more severe impairment of the organ.

The non-pathogenic RPS strain of E. histolytica that was used as control in this study was unable to produce lesions, or even to infect the animals, whether or not of S. mansoni was present, thus showing that mixed infection could not convert this strain into a pathogenic type.

Although Knight and Warren\textsuperscript{10}, using mice as experimental models, demonstrated that previously existing schistosomiasis was a risk factor for the seeding of intestinal amoebiasis, in the present study cecal inoculation of trophozoites in rats did not show lesions in these animals, or even in the presence of S. mansoni granulomas (data not shown). The resistance of these animals to intestinal infection with trophozoites of E. histolytica was also observed by Ghadirian and Meerovitch\textsuperscript{7}. However, the more diarrheic cecal content and the presence of trophozoites in the animals presenting coinfection lead us to wonder about the possibility that lesions might form if a longer period of association were studied. In these animals, thickening of the mucosa could be observed, caused by granulomas that led to formation of an area of constriction of the cecal lumen, with abundant mucus and presence of necrotic tissue. This created an environment to enable the maintenance of E. histolytica at this site for a longer period. This could explain the high prevalence of amoebiasis detected in endemic regions for schistosomiasis\textsuperscript{1 8 12}. In the animals inoculated with E. histolytica only, the presence of trophozoites could not be observed during examination of the cecal contents, probably due to the normal intestinal motility of the organ, as well as the absence of S. mansoni granulomas, which could alter the intestinal peristalsism of the animal.

The initial hypothesis was that the presence of S. mansoni granulomas would facilitate E. histolytica infection, since there would be a change in the hepatic microenvironment and exposure of the extracellular matrix and its components\textsuperscript{8}. Moreover, collagen resulting from S. mansoni granulomas might serve as adhesion sites for subsequent amoebic colonization. Nevertheless, adhesion of trophozoites on S. mansoni granulomas was not observed in the histological sections. However, exacerbation of tissue damage was observed when both diseases were present,
which resulted in a higher percentage of deaths among the animals, which suggests that there is an additive effect between the typical lesions of each disease.

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REFERENCES