Morphological Aspects of *Schistosoma mansoni* Adult Worms Isolated from Nourished and Undernourished Mice: a Comparative Analysis by Confocal Laser Scanning Microscopy

Renata Heisler Neves/++, José Roberto Machado-Silva/+/++/+++, Marcelo Pelajo-Machado*, Sheila Andrade Oliveira, Eridan M Coutinho**/+++, Henrique Leonel Lenzi*/+++, Delir Corrêa Gomes***/+++**

Departamento de Patologia e Laboratórios, Faculdade de Ciências Médicas, Uerj, Av. 28 de Setembro 87, fundos, 20551-031 Rio de Janeiro, RJ, Brasil *Departamento de Patologia ***Laboratório de Helmintos Parasitos de Vertebrados, Departamento de Helmintologia, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brasil **Departamento de Imunologia, Centro de Pesquisa Aggeu Magalhães-Fiocruz, Recife, PE, Brasil

Malnutrition hampers the course of schistosomiasis mansoni infection just as normal growth of adult worms. A comparative morphometric study on adult specimens (male and female) recovered from undernourished (fed with a low protein diet – regional basic diet) and nourished (rodent commercial laboratory food, NUVILAB) white mice was performed. Tomographic images and morphometric analysis of the oral and ventral suckers, reproductive system and tegument were obtained by means of confocal laser scanning microscopy. Undernourished male specimens presented smaller morphometric values (length and width) of the reproductive system (first, third and last testicular lobes) and thickness of the tegument than controls. Besides that, it was demonstrated that the dorsal surface of the male worms bears large tubercles unevenly distributed, but kept grouped and flat. At the subtegumental region, vacuolated areas were detected.

It was concluded that the inadequate nutritional status of the vertebrate host has a negative influence mainly in the reproductive system and topographical somatic development of male adult Schistosoma mansoni, *inducing some alterations on the structure of the parasite.*

Key words: *Schistosoma mansoni* - undernutrition - confocal scanning laser microscopy

Schistosomiasis is a very widespread disease in the developing world and is one of the most important helminth infections. Malnutrition and schistosomiasis mansoni are both serious public-health problems in Northeast Brazil and often overlap in the same geographical area (Coutinho et al. 1997a). Meanwhile, the role played by the nutritional status of the host on the development of schistosomiasis and vice versa is not clearly understood (Ferreira & Coutinho 1999).

The relationships between undernutrition of the host and parasite infection have been studied in the murine model. Based on food habits of Northeast Brazilian population, a regional basic diet (RBD) that is a deficient food blend with low protein-content, was produced and assayed in the acute phase of murine schistosomiasis. This diet had a negative effect on growth, food intake, reduced protein absorption and failed to develop hepatic periportal fibrosis in infected animals (Coutinho et al. 1997b).

It has been reported that the parasitic load isolated from mice fed with a deficient diet was decreased, both adult male length and number of testicular lobes also underwent reduction (Magalhães et al. 1986). However, it is not known if structural alterations in the reproductive system and in other topographical areas of the helmith body can also occur. In order to clarify this issue, entire specimens of *Schistosoma mansoni* were analyzed by confocal laser scanning microscopy (CLSM) according to procedures previously published (Machado-Silva et al. 1998).

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+Corresponding author. Fax: +55-21-587.6112. E-mail: machado@uerj.br
++Research fellows Faperj, proc. no. E-26/151.659/99
+++Research fellows CNPq, proc. nos 523656/96-3, 380845/99-7, 351223/97-5 and 303124/89-1, respectively
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MATERIALS AND METHODS

Swiss Webster *Mus musculus* were percutaneously infected with 80 cercariae (BH strain) shed from *Biomphalaria glabrata*. Undernourished animals were fed with a multivariate and essentially low protein diet (RBD) and compared to well fed control mice (NUVILAB diet). Seventy days post-infection, animals were killed, and adult worms were recovered by conventional perfusion technique. Specimens of both sexes were fixed in 10% formalin, stained with carmin chloride, cleared with beechwood creosote and mounted in a mixture of Canada balsam and creosote 1:1 (Machado-Silva et al. 1994). Whole-mounted parasites were analyzed by CSLM (LSM 410, ZEISS) in DIC and/or reflected mode, using 543 He/Ne laser, with LP 570 filter. The images were transferred from the LSM computer to Microsoft Imager^{TM} and Corel Draw 6.0^{TM} for the final adjustments of contrast, brightness and gamma correction and then printed in a Codonics NP 1600^{TM} printer (Machado-Silva et al. 1998). Tomographic images of the oral and ventral suckers, reproductive system and tegument were obtained. Morphometric analysis (length and width) of the first, third and last testicular lobes and tegument was performed using a confocal system (Carl Zeiss LSM). The data are analyzed by the statistical program SPSS. Statistical significance was determined by using Mann-Whitney and significance was determined with the use of p values < 0.05.

RESULTS

Morphometric values of male specimens from undernourished hosts were lower than in the controls. The mean values of testes length were 64.06 ± 4.80 µm and 87.98 ± 10.08 µm, and of testes width, 54.93 ± 1.33 µm and 60.30 ± 8.18 µm, respectively. Average measurements of the tegument thickness were 11.42 ± 1.90 µm and 15.86 ± 6.78 µm for undernourished and well-fed animals, respectively. Only testes length was statistically significant ($p < 0.05$).

Specimens recovered from well-nourished or undernourished mice did not show morphological alterations in the suckers. In the undernourished worms, the dorsal surface of the male worms bore large tubercles, kept grouped and flat contrasting with well-fed specimens. Although the tubercles observed in undernourished animals seem to be larger and more numerous than those observed in the well fed mice, they are, in fact, flat and concentrated (Figs 1, 2). The subtegumental region presented vacuolated areas (Figs 3, 4). Male specimens exhibited a single testis composed by combining lobes. However, parasites recovered from undernourished animals exhibited fewer differentiated cells in the testicular lobes than those from well-nourished ones.

In both groups of mice (nourished and undernourished), each female worm showed oocytes with different sizes and only one egg in different stage of maturation along the oviduct. No morphometric differences were found in uterine egg between female parasites isolated from both groups of mice (Figs 5, 6).

The changes described for male and female worms were uniform in all specimens examined.

DISCUSSION

The topographic aspects of the suckers were in agreement with previous report based on a ultrastructural study by scanning electron microscopy (Machado-Silva et al. 1997).

A host environment adverse to schistosome somatic development occurs in several conditions: nutritional state of the host (Akpom 1982), host hormones (Mendonça et al. 2000) and lack of interleukin-7, leading to dwarf parasites (males and females) (Wolowczuk et al. 1999). Nevertheless, it is not clear how those factors interact with the parasite (Mendonça et al. 2000). The physiological relationship between a parasite and its host is complex and the details of several biochemical processes should still be determined.

The tegument is an important interface between *S. mansoni* and its environment in the host because adult worms perform adjustable mechanisms for their survival and resistance to immune response (Abath & Werkhauser 1996). No information is available concerning a presumed correlation between thickness of the tegument and physiological conditions of the host. Probably, these morphological alterations seen in the undernourished worms are consequence of their smaller size since they do not succeed to reach a full developed stage in deficient hosts (Magalhães et al. 1986). By means of scanning and transmission electron microscopic studies the extent of these morphological alterations could be better evaluated.

It was concluded by this study with CLSM that the nutritional status of the vertebrate host has a negative influence on the somatic development of male and female *S. mansoni* adult worms, inducing some quantitative and qualitative alterations in the parasite structure.

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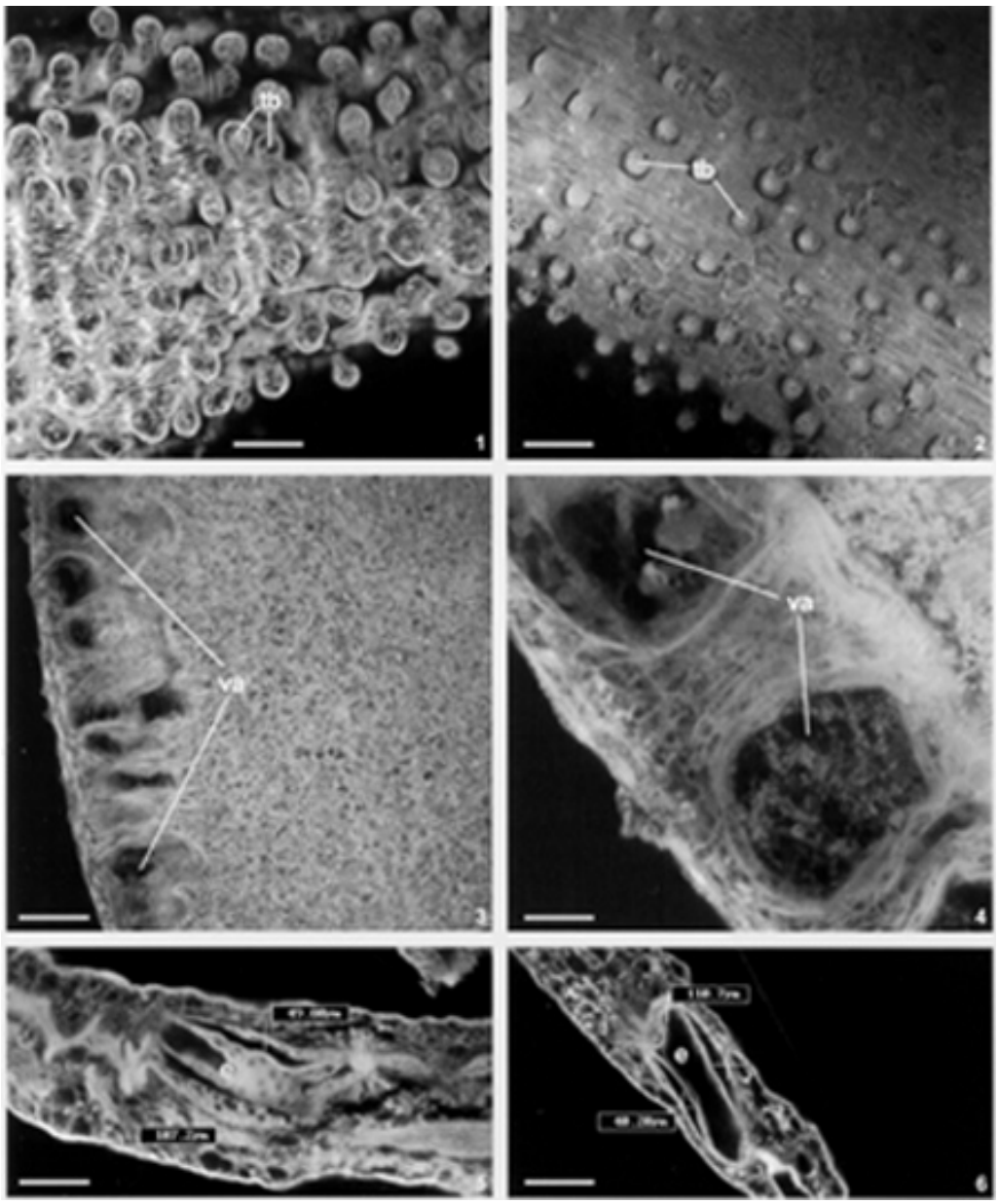

Schistosoma mansoni - Fig. 1: tubercles of undernourished animal. Fig. 2: tubercles of well-fed animal. Fig. 3: vacuolated area of undernourished animal. Bar common to Figs 1-3 = 45 µm. Fig. 4: vacuolated area of undernourished animal, detail. Bar = 3.28 µm. Fig. 5: uterine egg of undernourished animal. Bar = 38 µm. Fig. 6: uterine egg of well-fed animal. Bar = 45 µm

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