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Murine Model of Neuroschistosomiasis Mansoni: Clinical, Histological and Magnetic Resonance Imaging Studies

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Abstract

The *schistosomiasis mansoni* infection is responsible for 3.6% of the worldwide estimated causes of death and the central nervous system can be affected. In humans, the eggs of this helminth have been found in the leptomeninges, cerebral cortex, basal ganglia, choroid plexus, cerebellum and spinal cord. Neurological manifestations, histhology and magnetic resonance imaging of neuroschistosomiasis mansoni in humans serve as our chief reference points for the examination of the experimental infections in murine model. In this study, experimental infection of *S. mansoni* cercariae in mice aims to demonstrate the presence of granulomas in the brain and correlate to the clinical, histologic, and magnetic resonance findings. Twenty five *Swiss-webster* mice were infected subcutaneously, and followed for 160 days post-infection. Another group of twenty five mice were not infected and kept as controls. Images were obtained in the different planes by magnetic resonance. Histological samples were stained by Hematoxilin and Eosin (HE) to examine *S. mansoni* eggs, granulomas and inflammatory lesions. The results showed neurological manifestations as head and chest tilt (to the left or right side), hemiparesis, ataxia, body contortion, loss of balance and spinning, induced by granulomas in several regions of the central nervous system, and vascular changes associated with haemorrages. The MRI indicated multiple irregular nodules dispersed associated with oedema. These findings indicate that the murine model subcutaneously infected by *S. mansoni* cercarie may be used for studying mechanisms leading to human neuroschistosomiasis.

Keywords: Schistosomiasis; *Schistosomiasis mansoni*; Neuroschistosomiasis murine model; Encephalitis; Neuroinfection

Introduction

The World Health Organization estimates that between 200 and 300 million people worldwide are infected with Schistosoma spp. and that 800 million people in the world are at risk of infection. In Brazil, approximately 2.5 million people are infected with *Schistosoma mansoni* and 30 million are exposed to infection [1,2].

Central Nervous System (CNS) involvement in schistosomiasis can occur during acute primary infections, but as the disease becomes chronic, neurological complications can occur as the newly forming of granulomas are smaller, shrunken and fibrotic [2,3].

In the year of 1944, 800 people from Asia continent were attended at Moore General Hospital (North Caroline-United States of America) and approximately 2% (160 people) developed cerebral complications attributed to schistosomiasis japonica [4-8]. In Zimbabwe, Gelfand (1950) found *S. haematobium* eggs in the digested brains of 56% (28 people) of 50 patients with *S. haematobium* infection of the bladder. Alves (1958) found that in 28% of 150 unselected autopsy cases *S. haematobium* eggs were detected in the brain [9-11]. It is estimated that the incidence of neurological complications varies between 0.3% and 4% of *schistosomiasis mansoni* [2]. The incidence of encephalic demage caused by *S. mansoni* in humans is unknown.

Neurological manifestations of schistosomiasis are caused by an increase in intracranial pressure, and the focal signs are triggered by the tumor masses produced by granulomas, often in the productive phase with slight fibrosis, which suggested the chronic phase of infection. The initial signs and symptoms include headache, focal or generalized seizures, ataxia, nystagmus, nausea and vomiting, intracranial hypertension and various neurological deficits [2].

For many years, we have observed evidence of brain disease (hemiplegia, spinning and urinary retention) in mice infected with *S. mansoni*, but these mice were considered to have other diseases, such as labyrinthitis or cerebral infection [2].

Thus, neuromotor manifestations presented by infected animals should characterize neurological damage caused by *S. mansoni* eggs in the murine model. Therefore, the relationship between histological findings and the neurological signs of encephalic involvement, such as hemiparesia, spinning, head tilt, chest tilt, ataxia, and loss of balance could be established in this study.

Aloe et al. described eggs, both with and without granuloma formation, in CD-1 mice infected with 60 *S. mansoni* cercariae (Puerto Rican strain). However, the mice did not present the signs of brain disease. Additionally, Silva et al. observed very few eggs in the brains of

small percentages of BALB/c, C57BL/6 and *Swiss* albino mice that were infected once or five times with 30-50 cercariae (Feira de Santana strain) and the authors concluded that the model was not suitable for studying neuroschistosomiasis [2,3,12].

Magnetic Resonance Imaging (MRI) is a non-invasive diagnostic technique that can readily detect gross features of brain tissue in order to exclude neurosurgical causes such as tumor or stroke. As observed in humans affected by brain schistosomiasis, we decided to apply, in this research, the MRI technics to verify the lesions associated to encephalic neuroschistosomiasis in the mice model.

Thereby, such experimental model may allow the identification of pathways of the eggs or pairs of parasites can reach the CNS, the post-infection time for the occurring neuromotor manifestations and the topography of the histological lesion involved (Figure 1).

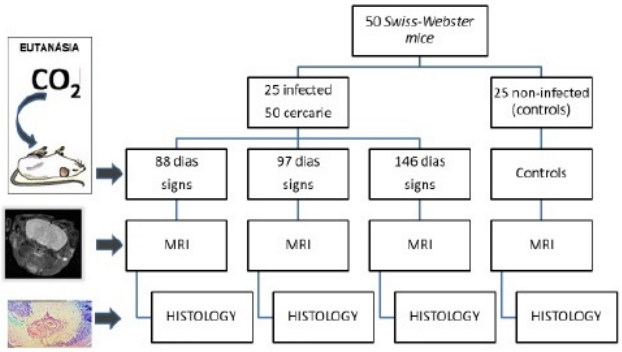

Figure 1: Experimental Essay.

Given these limited findings, we decided to further investigate murine encephalic neuroschistosomiasis mansoni, trying to answer the following questions: (1) Identify the topography and granulomatous lesion; (2) Does the presence of brain granulomas in mice cause neuromotor deficits as described in humans; (3) Correlate the neurological manifestations to the sites of brain injury; (4) To describe the changes in the magnetic resonance image of the brain and to correlate with the encephalic lesions.

Our present findings indicate that chronic *schistosomiasis mansoni* in mice results in the neuromotor behavior signs, granulomatous lesions in the CNS proved by magnetic resonance imaging in subcutaneously infected mice.

In summary, the present study reproduces in the *Swiss-webster* murine model an encephalic neuroschistosomiasis mansoni, establishing it fundamental in the researches of pseudotumor pathogenesis, in the analysis of cytokines and chemokines by Cerebro Spinal Fluid studies, diagnostic imaging methods of MRI and medical conduct for the benefit of human patients.

Materials and Methods

Mice and infection

Swiss-webster male mice, weighing approximately 20 g, were obtained from Oswaldo Cruz Foundation (Belo Horizonte, Brazil), and maintained under standard conditions. At 6 weeks of age, mice were infected subcutaneously with 50 cercariae of a LE strain of *S. mansoni*, maintained at the Rene Rachou Institute (Minas Gerais, Brazil) by passage in *Mus musculus* albino mice and in *Biomphalaria glabrata* snails. The origin and maintenance of the *S. mansoni* used in this study have been described previously by Pellegrino & Katz (1972). All mice, infected and control animals, were provided with food and water *ad libitum*, under the 12 h light/12 h dark cycle, temperature 22 ± 1°C and air humidity of 40–50%.

At 88, 97 and 146 days post-infection, euthanasia procedures was performed (n=2/group), by CO2 gas chamber, according to the guidelines and principles of the Brazilian Council on Animal Care and were approved by the local Institutional Animal Care Committees at the Federal University of Minas Gerais and the Rene Rachou Research Institute (FIOCRUZ/MG, Brazil). The *ex vivo* samples had a catheter placed into the right heart and perfused by a fixative solution of 10% paraformaldehyde (PFA). The worm recovery was carried out as per the technique prescribed by Pellegrino and Siqueira [13-18].

Clinical monitoring

SHIRPA protocol for phenotype assessment designed as a series of individual tests that gives data regarding the integrated function of cortical arousal, cerebral locomotor control, and neuromuscular function. Such test-specific performance is directly comparable between animals, over time, and between groups. The locomotor parameters, such as head and body rotation, absence of escape reaction, paresis, loss of balance reflex, altered muscle tone, ataxia and spinning were applied [19].

Magnetic Resonance Imaging

Mouse heads were removed and immersed in 10% PFA for more than 48 h. To preserve the shape of the brains during imaging, the brains were left inside the skull. The experiments were performed on a 7T magnetic resonance scanner (MRI System 7T/210 ASR Horizontal Bore Magnet, Agilent Technologies). *Ex vivo* brain images were obtained using 3D T1 GRE (TR/TE: 370 ms/5 ms, MATRIX: 128x96x 96, FA: 35°, NEX: 13, FOV: 20x15x15 (mm), acquisition time: 12 h 18 min), coronal MULTI ECHO (TE/TR: 3000 ms/9 ms, 3 ECHOS, NEX: 30, MATRIX: 128×128, FOV: 15x15 (mm), SLICES: 30, SLICE THICKNESS: 0.5 mm, no GAP, acquisition time: 3 h 12 min).

For each dataset, the images were visually inspected for artifacts. For image processing, the MRIcro software (http://www.mccauslandcenter.sc.edu) was used to measure and compare the lesions dimensions with findings in histopathology.

Histological analysis

Imaging was done, brain and skull was immersed in 7% nitric acid for decalcification. After 1 day (24 h), the whole skull was sectioned in 3 mm thick slices, and dove in 7% nitric acid for 24 h for complete decalcification. After that, the fragments were sectioned in 1.1 mm thick slices, each one placed in a paraffin block (10-11 blocks for each animal). Serial 4 µm sections (obtained from 50 µm intervals between each) from all paraffin blocks were stained with Hematoxylin and Eosin (HE). For each animal, 18-20 g weighing, sections were examined in light microscope, looking for any morphological lesion, especially schistosoma eggs and/or granulomas.

The right hemisphere of each animal's skull was stained with Nankin® ink for identification.

Brain topography

Was performed using Allen's brain atlas, data portal (http://mouse.brain- map.org/static/atlas).

Statistical analysis

When significant differences were obtained, comparisons between groups were carried out by Fisher's test. Analyzes were performed in the STATA 12.0 software (Stata Corporation, College Station, Texas) at 5% level of significance.

Results

Neurological alterations appeared at 88, 97 and 146 days post-infection. Euthanasia occurred immediately after the emergence of the neurological signs.

Neurological manifestations

The neuromotor alterations were head and chest tilt (to the rigth or left side), paresis, imbalance reflexes, ataxia and rotational motion (spinning) (Table 1) (Figure 2).

Neurological Manifestations	Infected		Control		*p*
	n	%	n	%	
Spinning	3	12	0	0	0.235
Head tilt (right side)	2	8	0	0	0.49
Head tilt (left side)	1	4	0	0	0.999
Chest tilt (right side)	2	8	0	0	0.49
Right hemiparesis	1	4	0	0	0.999
Left hemiparesis	2	8	0	0	0.49
Ataxia	3	12	0	0	0.235
Imbalance	3	12	0	0	0.235

Table 1: Neurological manifestation observed in three mice infected with 50 cercarie in FIOCRUZ/Brazil laboratory, 2016-2017.

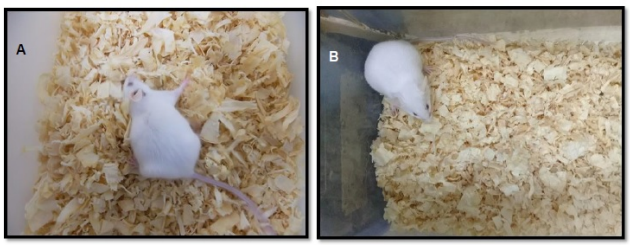

Figure 2: A. *Swiss Webster* mice infected with 50 cercarie of *S. mansoni*. The animal presented head tilt for the left side. B. Mice not infected (control), with habitual posture.

MRI evaluation

MRI images enhanced abnormalities in encephalic parenchyma of the symptomatic animals in comparison to the control mice images. The histopathological features present in these infected mice revealed the tumor mass caused by the granulomatous reaction (Figure 3). On MRI there were signs suggesting hemorrhages inside the brain (Table 2).

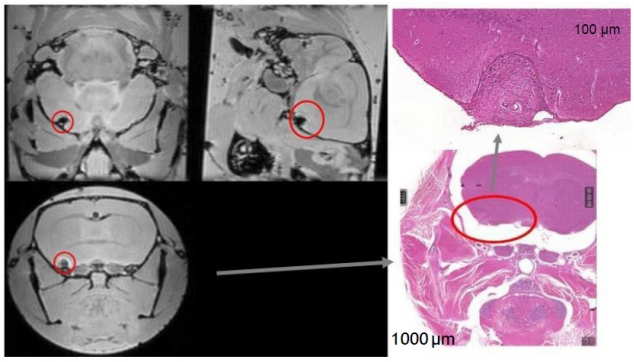

Figure 3: Serial brain slices from the brain of the mouse, show in lesions due to *S. mansoni*. T1-weighted sequences images. Nodular lesion surrounding the parasite egg (HE), located in the piriform area.

Topography	Infected		Control		*p*
	n	%	n	%	
Limbic lobe	2	8.0	0	0.0	0.490
Sensorialmotor area (cerebral cortex)	3	12.0	0	0.0	0.235
Visual area (cerebral cortex)	2	8.0	0	0.0	0.490
Auditory area (cerebral cortex)	1	4.0	0	0.0	0.999
Fissures of cerebellar cortex	2	8.0	0	0.0	0.490
Brainstem	2	8.0	0	0.0	0.490
III Ventrícle	1	4.0	0	0.0	0.999
IV Ventrícle	1	4.0	0	0.0	0.999
*Universidade Federal do Rio de Janeiro, Brazil.					

Table 2: MRI topography of encephalic lesions attributed to *S. mansoni* eggs and granulomatous reaction, of three infected mice subcutaneasly, CENABIO/UFRJ*, 2016–2017.

Histological brain topography

Morphological examination of serial coronal brain sections showed *S. mansoni* eggs or granulomas found in the olfactory tract, limbic lobe, cerebral cortex, cerebellum cortex, III and IV ventricles, and brainstem (Table 3). The granulomas were localized in proximity to arteries, suggesting that the ova gain access to the CNS *via* arterial system. Red blood cells were also observed in most brain areas.

Topography of Lesions	Infected		Control		*p*
	n	%	n	%	
Olfactory tract	2	8.0	0	0.0	0.490
Limbic lobe	3	12.0	0	0.0	0.235
Sensorialmotor area (cerebral cortex)	3	12.0	0	0.0	0.235
Visual area (cerebral cortex)	2	8.0	0	0.0	0.490
Audictory area (cerebral cortex)	1	4.0	0	0.0	0.999
Fissures cerebellar cortex	2	8.0	0	0.0	0.490
Brainstem	2	8.0	0	0.0	0.490
III Ventrícle	1	4.0	0	0.0	0.999
IV Ventrícle	2	8.0	0	0.0	0.490
*Universidade Federal de Minas Gerais, Brazil.					

Table 3: Topography of brain lesions attributed to *S. mansoni* eggs, Department of Pathological Anatomy/School of Medicine/UFMG*, 2016–2017.

Histological analysis

Eggs of *S. mansoni* were disseminated in the right and left cerebral hemispheres, in regions of the leptomeninges, cerebral and cerebellar parenchyma and brainstem. In the most of samples, the eggs reached the encephalon by arteriolar system (Figure 4).

Epithelioid granulomatous infiltrate were most frequent in the leptomeninges, fissures and ventricles, in the brain and cerebellum. *S. mansoni* eggs with low inflammatory reaction were common in the cerebral and cerebellar parenchyma. All the eggs observed had miracidium, proving recent deposition in the encephalon. Macrophages, eosinophils, lymphocytes identified the inflammatory cells. Fibroblasts and areas with collagen were also present (Figure 4).

Discussion

In 25 *Swiss-webster* mice subcutaneously infected with 50 cercariae of the *S. mansoni* (LE strain), three mice presented the following neurological manifestations: spinning, hemiparesis, head and chest tilt (to the right or left side), ataxia, loss of balance and body contortion. MRI sagittal, coronal and axial T1w images of brain demonstrate in encephalon bilateral focal hypersignal, indicating morphological changes. Histology confirmed the lesions in the brain. The samples presented viable eggs of *S. mansoni* near arterioles and red blood cells.

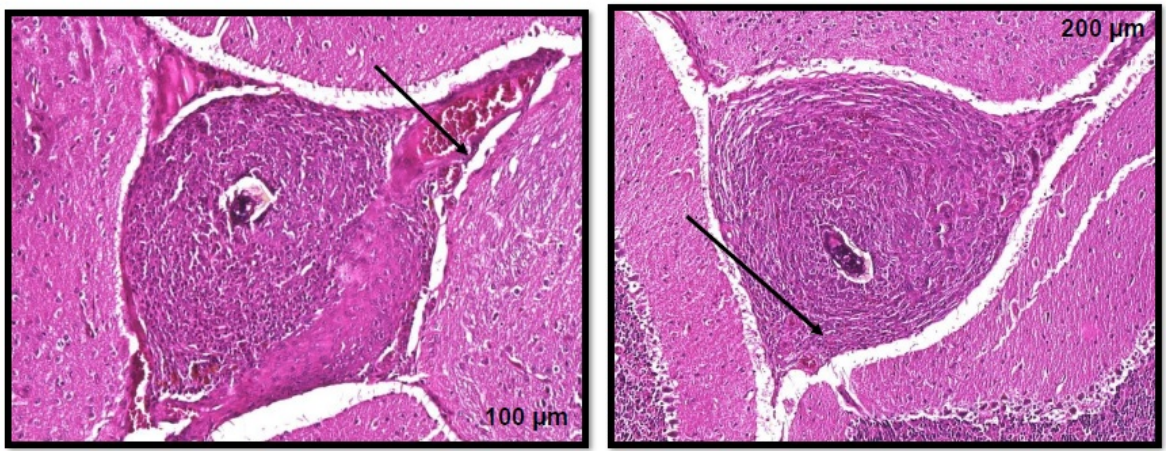

Figure 4: *S. mansoni*'s eggs in the brains tissue form simptomatic mice. Arrows show arteritis and fibrinoid necrosis.

This is the first description of relationship between brain lesions and neurological signs in *Swiss-webster* murine model infected subcutaneously with *S. mansoni* cercariae.

Experimental mansoni model of *Swiss-webster* mice was not suitable for experimental Neuroschistosomiasis [20]. However, the design of their study was based on the digestion of mice organs: the entire encephalus was digested in 60 ml of a 4% potassium hydroxyde solution for the counting eggs. Microscopic search of eggs were collected after sedimentation for several h. The design of our study differs entirely from the above reported. Moreover, mice presented neurological alterations after 88 days of infection in our experiment, whereas in the previous study, mice were sacrificed 31, 40 and 52 days after cercariae injection.

Animals were observed until 160 days post-infection. At that time, 3 mice (12%) presented neurological manifestations suggestive of brain injury. The first symptomatic animal was sacrificed at 88 days post-infection, the second at 97 days and the third at 146 days. The control animals of the respective symptomatic did not present neurological alterations.

Qualitative SHIRPA protocol was applied based on gradations by scores to track basic neurological functions [19]. Such protocol integrates the functions related to skeletal striated muscle, sensory systems, motor neuron, autonomic system and spinocerebellar pathways. The neurological manifestations are consistent with characteristic changes of Central Nervous System (CNS) functional damages.

Histo-topographic survey of the mice brain identified granulomatous lesions disseminated in the cerebral hemispheres, in Sensory-motor, visual and auditory cerebral cortex areas, cerebellum fissures, olfactory tract, III and IV ventricles, limbic lobe and

brainstem. Associated to granulomatous lesions were observed leptomeningeal thickening in cerebral and cerebellum cortex. The microscopic picture is characterized by granulomas constituted by macrophages, eosinophils, lymphocytes and giant cells arround the ova. Fibroblasts and focal collagen are also present (Figure 5).

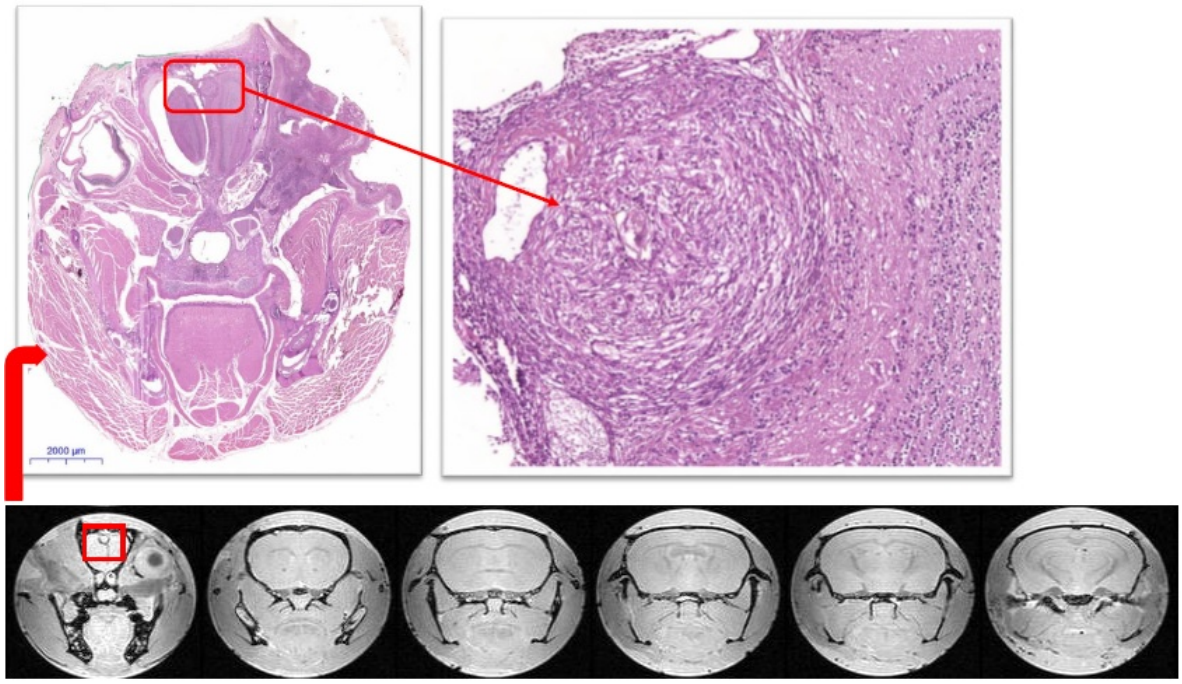

Figure 5: Histological slice of the olfactory tract (HE).T1-weighted sequences images showing the MRI lesion.

Aloe et al. [3] described schistosome eggs in histological sections from the brain of mice and reported that mice with *S. mansoni* periovular granulomas in the brain showed decrease of nervous growth factor expression [21]. Based on such findings, Fiore et al. reported that mice infected with *S. mansoni* exhibited behavioral disturbances, probably associated with modifications in the levels of nerve growth factor and cytokines induced by granulomas [3,21,22]. These two reports did not indicate how frequently *S. mansoni* eggs reach the CNS of the mouse. There was also no sign of neurologic involvement.

The MRI image suggested haemorrhages in our samples. There are no reports about MRI image that correlate with brain lesions by encephalic neuroschistosomiasis mansoni in the murine model (Figure 4).

Haemorrhages in the subarachnoid and intraparenchymal in the cerebral and cerebellar regions were identified by histological samples. The proximity of eggs and granulomas of *S. mansoni* to the arterioles suggests that the penetration into the CNS in the murine model occurred *via* the arteriolar route. Often the arteriole wall presented an inflammatory pattern (arteritis) of fibrinoid necrosis (Figure 3). Human brains were identified within the cerebral arteries *S. mansoni* eggs surrounded by mononuclear inflammatory cells. Herein we observed segmental ruptures and alterations of the epithelium with arterial fibrinoid necrosis [23-24].

Conclusions

In summary, the present study demonstrates that the subcutaneous infection in *Swiss-webster* mice by *S. mansoni* cercariae develops a neurological disease quite similar to the neuroschistosomiasis described in humans.

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