RESUMO

Histopatologia da infecção por *Fasciola hepatica* em *Meriones unguiculatus*

Três grupos de 60 *Meriones unguiculatus* foram inoculados, respectivamente, com 3, 5 e 8 metacercárias de *Fasciola hepatica* e dez animais permaneceram como controle. Com intervalo de dez dias, seis animais do grupo infectado e um animal do grupo controle foram eutanasiados, sendo coletadas amostras para a histopatologia dos órgãos internos. Foram observadas áreas hemorrágicas, focos de necrose e fibrose no fígado, no rim direito e em órgãos internos contidos nas cavidades torácica e abdominal em 20,6% dos animais infectados, independentemente do número de metacercáreas. O fígado foi o órgão mais lesado, indicando o tropismo que o parasito apresenta durante seu desenvolvimento. Foram observadas alterações no espaço porta-hepático, dilatação do ducto biliar e de ramos da artéria hepática, rompimento de ramos da veia hepática e hemorragia periportal. Foram recuperados parasitos e ovos de *F. hepatica* aderidos à parede interna do ducto biliar. A análise histopatológica do fígado e do linfonodo mesentérico mostrou a presença de hemossiderose e de reação inflamatória macrofagocitária. No fígado, pulmões, diafragma, baço, linfonodos mesentéricos, rins, pâncreas e intestino delgado, havia infiltrado inflamatório misto formado por polimorfonucleares e mononucleares, áreas hemorrágicas, e focos de necrose e fibrose. Os resultados indicam que *M. unguiculatus* mantém a infecção por longos períodos, permitindo também o desenvolvimento do parasito em diferentes órgãos.

DESCRITORES: *Fasciola hepatica.* Histopatologia. *Meriones unguiculatus.*

INTRODUCTION

The development of *Fasciola hepatica* in different experimental host species has been reported in different parts of the world (11, 12). The descriptions by Dawes (3) are among the first reports of development of *F. hepatica* in a definitive host. The use of mice for evaluating the migration and feeding of the parasite after penetration into the liver led to a detailed description of the different aspects of *F. hepatica* pathogenesis (5, 6, 7). However, in this model, parasite maturation usually causes death. Rats and rabbits represent suitable models to study the therapeutic and immunological host responses to *F. hepatica* infection (1). Most studies related to the pathogenesis of fascioliasis focus on the hepatic changes. Reports of injuries in other organs and tissues are quite rare (13), and therefore, it is essential for a suitable laboratory model to permit the complete investigation of *F. hepatica* pathogenesis as it migrates into the thoracic and abdominal cavities before penetrating the liver. In the search for a suitable experimental model for *F. hepatica*, Helfer & Knapp (10) showed the susceptibility of *Meriones unguiculatus* to infection and reported only hepatic lesions that were similar to those found in other animal models up to 50 days after infection (dpi).

The aim of the present study is to investigate the use of *M. unguiculatus* as a model to evaluate the pathological changes in the liver and other organs in the thoracic (lungs and diaphragm) and abdominal cavities (spleen, kidneys, pancreas, small intestine and mesenteric lymph nodes) during the development of the parasite up until 100 dpi.

MATERIAL AND METHODS

Infection

One hundred ninety, ten-week-old *M. unguiculatus* individuals (of both sexes, with an average weight of 60 g) were used in this study. The animals were randomly divided into 4 groups. Three groups (I, II, and III) of 60 animals were orally inoculated with 3, 5, or 8 metacercariae, each suspended in dechlorinated water and inoculated with 250 µl volumetric pipette. The control group consisted of 10 uninfected animals that orally received the same volume of saline solution. The metacercariae were obtained by experimental infection of 300 specimens of *Lymnaea columella* grown and maintained by the Laboratory of Veterinary Helminthology, Institute of Biological Sciences, Federal University of Minas Gerais.

Post mortem examinations

In every 10-day interval, from the tenth to the 100th dpi, 6 animals from the infected groups and 1 animal from the control group were euthanized by cervical dislocation according to the Resolution 714 of the Federal Council of Veterinary Medicine. The thoracic and abdominal cavity of each animal was flushed with 0.85% saline solution. The viscera (lung, liver, spleen, kidneys, pancreas, small intestine and mesenteric lymph nodes) were removed and transferred to Petri dishes containing the same solution. After macroscopic evaluation, fragments with lesions were removed, fixed in 10% formalin, and processed according to the routine technique for paraffin embedding. Four micrometer sections were stained with Hematoxylin-Eosin (H&E), Gomori Trichrome, and Prussian Blue for histopathological evaluation.

RESULTS

Thirty-seven out of the 180 infected animals (20.6%), independent of the inoculum, showed gross and microscopic lesions in one or more organs in the thoracic and abdominal cavity. In group II (inoculated with 5 metacercariae), 15 animals (25.0%) showed gross and microscopic lesions. In groups I and III (inoculated with 3 and 8 metacercariae, respectively), lesions were found in 11 animals (18.4%), (Table 1).

Animals studied at 20, 30, and 40 dpi showed a higher number of macroscopic and microscopic lesions than in other intervals. Grossly, the liver presented dark reddish petechial hemorrhages – more common in earlier sampling (10, 20 and 30 dpi) and/or focal whitish spots suggestive of necrosis and fibrosis, diffusely distributed on the cut and visceral surfaces (progressively increasing along

the intervals). Gallbladder was dilated and there were adhesions between the dorsal and ventral lobes of the liver.

Histopathological examination revealed track-like lesions varying from acute hemorrhagic necrosis to active granulomas with organized fibrotic areas generated by eggs and worms (imatures and adults) in the parenchyma of the liver and mesenteric lymph nodes. Most of the chronic lesions showed the presence of lymphocytes and macrophages. Track-like lesions were either necro-hemorrhagic (more frequent on the initial sampling) or chronic fibrotic in tortuous migratory parasitic pathways. Sometimes, they were filled with a mass of cellular debris. Bordering these areas were inflammatory infiltrates comprising polymorphonuclear cells, which were predominantly neutrophils and eosinophils. As the timing of the experiment increased, increasingly numbers of mononuclear cells became evident.

Table 1. Macroscopic and microscopic changes in the organs where eggs and parasites were found in the individuals of *Meriones unguiculatus* infected with *Fasciola hepatica* metacercariae examined at 10-day intervals up to 100 days after infection

Organ	N Mc	N of Animals +	Dpi	Adherence	Haemorrhage	Haemosiderosis	Inflammatory infiltrate	Fibrosis	Necrosis	Parasites	Eggs
Diaphragm	3	1	80	–	*	–	*		–	–	*
Liver	5	3	30	*	*	*	*	–	*	*	*
	5	2	40	*	*	–	*	–	*	*	*
	8	1	20	–	*	–	*	*	*	*	*
Kidney	8	1	40	–	*	–	*	*	*	–	*
Mesenteric lymph nodes	5	1	90	–	*	*	–	–	–	–	*
	8	1	20	–	*	*	*	–	–	–	*
Peritoneal cavity	5	1	40	–	–	–	–	–	–	*	*

N Mc, (Number of metacercariae); Dpi, (Days after infection); N of Animals +, (Number of positive animals); –, (Absence of macroscopic and microscopic alterations); *, (Presence of macroscopic and microscopic alterations)

Liver lesions mostly occurred around the portal spaces, with periportal hepatitis characterized by inflammatory infiltrates and dilatation of the bile ducts and branches of the hepatic artery in periportal spaces, sometimes with rupture of the portal vein and periportal hemorrhage (Plate 1, Fig. 1).

Parasites and eggs of *F. hepatica* were observed during the dissecation process, under a 10X magnification with a stereoscopic microscope on 20 and 40 dpi inside the cavity peritoneal, adhered to the parenchyma and to the inner wall of the bile ducts of the animals inoculated with 5 metacercariae.

At 80 dpi, hemorrhagic areas filled with hemosiderin were identified in the liver of the animals inoculated with 3 metacercariae. The surrounding inflammatory infiltrates predominantly consisted of polymorphonuclear in earliest sampling (10, 20 and 30 dpi) and then of mononuclear cells, which increased progressively along the evolution of the experiment.

Gross hemorrhagic spots were observed on the external and cut surface of the lungs, regardless of the number of metacercariae in the inoculums and of the time interval. Histopathological examination of these lesions in animals inoculated with 5 metacercariae revealed multifocal areas of hemosiderosis and inflammatory infiltrates with mononuclear cells, mainly lymphocytes and macrophages.

Similar findings were observed in other organs. Spleen showed red spots in the cut and external surfaces, sometimes with whitish areas. Microscopically red pulp had dilated capillaries (hyperemia) and even follicles had red blood cells within (hemorrhagic white pulp), besides multifocal necrosis and fibrosis. Besides that, there were also areas with thickened capsule, parenchymal disrupture and inflammatory infiltrates with polymorphonuclear and mononuclear cells around, hemorrhagic areas, sometime with fibrin deposition.

Mesenteric lymph nodes showed reddish spots in external and cut surface, suggestive of intense hemorrhages. Histopathological examination of the lymph node of an animal inoculated with 3 metacercariae at 80 dpi revealed hemosiderosis, hemorrhages and intense inflammatory reaction with macrophages and lymphocytes. *F. hepatica* eggs were found adhered to the capsule on the surface as well as within the parenchyma (Plate 1, Fig. 2). The diaphragm of the same animal had pinpoint reddish spots (petechias) on macroscopic examination. Eggs were detected between the myocytes, with a few surrounding macrophages and involved by a fibrotic reaction (Plate 1, Fig. 3).

Serosal surfaces of the small intestine were generally edematous, with a yellowish jelly aspect. Microscopically, besides the edema; small foci of hemorrhages and chronic infiltrates of mononuclear cells were distributed on the walls. One animal inoculated with 5 metacercariae had reddish areas of hemorrhages on the surface of the pancreas at 40 dpi.

The right kidney of an animal inoculated with 8 metacercariae showed reddish and whitish spots on external and cut surface. Microscopically, multifocal hemorrhages and necrosis with fibrosis were observed, besides an intense inflammatory reaction with polymorphonuclear cells, macrophages, and lymphocytes. *F. hepatica* eggs were found adhered to the tissue surrounded by inflammatory infiltrates (Plate 1, Fig. 4).

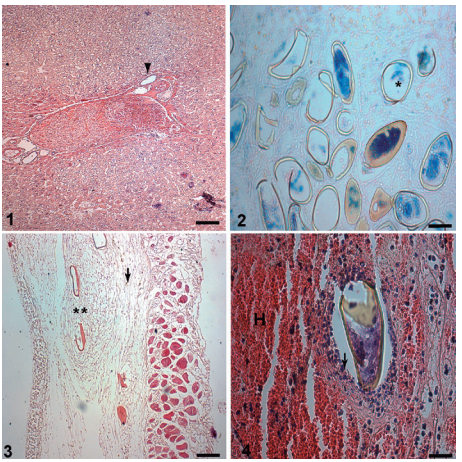

Figure 1. Micrograph of a liver specimen, at 20 dpi showing dilatation of the bile ducts (arrow had). H&E, bar = 100 µm

Figure 2. Micrograph of a mesenteric lymph node, at 80 dpi with several eggs of *Fasciola hepatica* adhered within the parenchyma (*). Prussian Blue, bar = 30 µm

Figure 3. Micrograph of a diaphragm at 80 dpi, with eggs of *Fasciola hepatica* adhered to the tissue (arrow) and macrophages surrounding a discrete fibrotic reaction (**). Gomori Trichrome, bar = 100 µm

Figure 4. Micrograph of a right kidney at 40 dpi, with an egg of *Fasciola hepatica* surrounded by an inflammatory infiltrate of polymorphonuclear and mononuclear cells, focal necrosis (arrow) and hemorrhage (H). H&E, bar = 30 µm

DISCUSSION

Lesions in liver, lungs, spleen, mesenteric lymph nodes, kidney, pancreas, and small intestine seemed to reflect the mechanical trauma caused by *F. hepatica* during their migration and feeding in these organs.

The results of our study revealed necro hemorrhagic lesions initially surrounded by acute inflammation, with polymorphonuclear cells in infiltrates, progressing to fibrotic and chronic inflammation with mononuclear cells in the different organs in the peritoneal cavity of *M. unguiculatus.* These findings indicate that the parasite can migrate and develop in organs other than just the liver, as is the case of the infection in other small animals (3, 5, 6, 7, 9, 14).

The microscopical lesions, characterized by necrotic areas filled with cellular debris and parasites, with severe hemorrhage in the spleen and in the mesenteric lymph nodes, suggest that *F. hepatica* can also affect lymphoid organs. Photomicrographs previously described in studies of Dawes (4) showed the presence of lymphoid cells in the caecum of the immature worms. According to the author, the cells of the lymphoid organs are small and easily broken with the use of an oral sucker, forming a homogenate, which is sucked up by the parasite. The changes in the lymphoid organs such as the spleen and lymph nodes at 80 and 90 dpi

in our study corroborates the observations of Dawes & Hughes (7) suggesting the passage and permanence of the parasite on other tissues for nutrients.

In this study, we found eggs of *F. hepatica* in the diaphragm, right kidney, and mesenteric lymph nodes, which seems to support the passage and presence of adult parasites in the other organs in the cavity. Also, these findings suggest that in this host, *F. hepatica* may migrate and feed in the liver and return to the peritoneal cavity. Migration and feeding on tissues other than hepatic tissues may not necessarily characterize ectopic migration. This behavior suggests that the parasite uses strategies that enable its survival.

The lesions observed in the liver of *M. unguiculatus* in our study were similar to those described by Helfer & Knapp (10). However, these authors did not report any changes in other organs. Their experiment ended at 55 dpi, as no living animals survived after that. In our study, *M. unguiculatus* maintained infection for up to 100 days, which was the clinical endpoint of the experiment. This observation suggests that *M. unguiculatus* can maintain infections for long periods. This can be attributed to the parasitic load used in this experiment, which may have contributed to animal survival. Determining the parasitic load to be used while infecting small laboratory animals is a key experimental criterion. According to Sinclair (13), several factors are associated with the parasitic load in *F. hepatica* infection: the purpose of the study and the type of vertebrate host to be used are important. In our study, there were no significant differences between the level of the lesions and the parasitic loads used. The recovery of parasites and a greater number of animals with gross and microscopic lesions at 20, 30, and 40 dpi suggest that these experimental intervals are adequate for the recovery of parasites, eggs, and for studying the pathological changes that occur during the development of *F. hepatica*.

The presence of inflammatory infiltrates around the necrotic lesions and parasite eggs are consistent with the reports of several authors (3, 8, 11, 13, 14). The inflammatory response to *F. hepatica* in *M. unguiculatus* in our study was characterized initially by the presence of polymorphonuclear cells and progressed to mononuclear cells after more prolonged stages of infection. Our results also reveal that *M. unguiculatus* presents a non-specific inflammatory response to *F. hepatica* infection. In general, the host responds with an intense cellular inflammatory response described in detail by other researchers (1, 2, 3, 4, 5, 8), where the migratory pathways generated by the parasites are filled with cellular debris and bordered by polymorphonuclear and mononuclear cells.

According to Dow et al. (8), macrophages and fibroblasts gradually occupy the injured areas, forming a fibrotic scar tissue, promoting a prolonged migration and further damage to the liver parenchyma. The repair of liver lesions was characterized initially by the fibrin deposition and later by fibrous scarring. In the spleen such lesions were also observed, at different intervals and, independent of the number of metacercariae in the inoculums. This may be consistent with active

migration into other organs, corroborating the reports that described the migratory behavior of the parasite in small laboratory animals.

The characteristic lesions of fascioliasis in *M. unguiculatus* observed in our experiments had similarities to those reported in experimental fascioliasis in mice (3, 4, 5, 6), rabbits (15), rats (14), sheep and cattle (2, 8). However, *F. hepatica* infection of *M. unguiculatus* had some peculiarities: the migration of the parasite to other tissues may have contributed to the maintenance of infection for prolonged periods. Such migratory behavior suggests a strategy as an escape mechanism that favors parasite survival. Although the lesions were evident only in 20.6% of the inoculated animals, in our experiments, *M. unguiculatus* maintained the infection for up to 100 days, suggesting that host may constitute an appropriate animal model for studying chronically *F. hepatica*-induced injuries. Studies on the development of *F. hepatica* in small laboratory animals, especially after prolonged periods of infection, may help understand the relationship between disease pathogenesis, host immune response, and parasite escape mechanisms.

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