

Autochthonous infection of buffaloes and cattle by *Fasciola hepatica* in Minas Gerais, Brazil

Infecção autóctone de búfalos e bovinos por *Fasciola hepatica* em Minas Gerais, Brasil

Ruth Massote Dracz¹; Walter dos Santos Lima¹

¹Laboratório de Helminologia Veterinária, Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, MG, Brasil

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Abstract

Fasciola hepatica is a digenetic trematode that parasitizes the bile ducts of different species of vertebrate hosts. In Brazil, this parasitosis is expanding and occurrences have been reported in the southern, southeastern, central-western and northeastern regions. This study aimed to report the first case of naturally infection of buffaloes by this parasite in the state of Minas Gerais. A total of 250 stool samples, 176 from cattle and 74 from buffaloes, from the districts of São José da Lapa and Pedro Leopoldo, were examined. Also, 402 snails of the genus *Lymnaea* were collected on the same farms and the viability of the eggs from naturally infected buffaloes and cattle and the susceptibility of the snails to infection were tested. A total of 54 animals were found to be positive, 33 cattle (18.75%) and 21 buffaloes (28.37%), and two molluscs showed immature forms of *F. hepatica*. In experimental infection of specimens of *Lymnaea*, cercariae were obtained through spontaneous elimination from the 57th day post-infection onwards. The importance of this first case report can be emphasized because it confirms that this parasite is being dispersed in municipalities in this state that had previously been considered to be unaffected.

Keywords: *Fasciola hepatica*, buffaloes, epidemiology, cattle.

Resumo

Fasciola hepatica é um trematódeo digenético que parasita os ductos hepáticos de diferentes espécies de hospedeiros vertebrados. No Brasil, essa parasitose encontra-se em expansão e a sua ocorrência tem sido descrita nas regiões Sul, Sudeste, Centro-oeste e Nordeste. Esse trabalho objetivou relatar o primeiro caso de búfalos naturalmente infectados no estado de Minas Gerais. Um total de 250 amostras de fezes, sendo 176 de bovinos e 74 de búfalos, provenientes dos municípios de São José da Lapa e Pedro Leopoldo foram examinadas. Também foram coletados 402 moluscos do gênero *Lymnaea* nas propriedades estudadas e a viabilidade dos ovos oriundos dos búfalos e bovinos naturalmente infectados e a susceptibilidade do molusco a infecção foi testada. Um total de 54 animais apresentavam-se positivos sendo 33 bovinos (18,75%) e 21 bubalinos (28,37%) e dois moluscos apresentaram formas imaturas de *F. hepatica*. Na infecção experimental dos exemplares de *Lymnaea*, foram obtidas cercarias eliminadas espontaneamente a partir do 57º dia pós infecção. Ressalta-se a importância desse primeiro relato de caso, pois certifica a dispersão do parasito em municípios antes considerados indenes no Estado.

Palavras-chave: *Fasciola hepatica*, búfalos, epidemiologia, gado.

Introduction

Fasciola hepatica (Linnaeus, 1758) is a digenetic trematode of the family Fasciolidae that shows cosmopolitan distribution and parasitizes the hepatic ducts of different species of vertebrate hosts. In Brazil, occurrences of this parasite have been reported in the southern, southeastern, central-western and northeastern regions, parasitizing cattle, sheep, buffaloes and wild animals such as capybara and capybaras (SILVA SANTOS et al., 1992; CUNHA et al., 2007;

BELLATO et al., 2009; LIMA et al., 2009; CARNEIRO et al., 2010; BENNEMA et al., 2014). Human cases have also been reported in the southern, central-western, southeastern, northeastern and northern regions, with the largest number of cases in the southern and southeastern regions respectively (MAS-COMA et al., 1999; PILE et al., 2000; OLIVEIRA et al., 2007).

Fasciolosis can cause major economic losses in parasitized herds because of reduced productivity of milk and meat, growth retardation in young animals, rejection of livers in slaughterhouses, abortions, mortality and expenditure on controlling this parasitosis (LIMA et al., 2009).

*Corresponding author: Walter dos Santos Lima
 Universidade Federal de Minas Gerais – UFMG, Av. Presidente Antônio Carlos, 6627, CEP 31270-901, Belo Horizonte, MG, Brasil
 e-mail: wlima@icb.ufmg.br

The increasing geographical dispersion of *Fasciola hepatica* in Brazil is due to trade and transfer of parasitized animals from areas where the disease is enzootic to locations that are unaffected but have favorable epidemiological conditions. Among these favorable conditions is the presence of snails of the genus *Lymnaea*, which are intermediate hosts for *F. hepatica*. Thus, such conditions also include the climatic and environmental factors that provide a suitable habitat for these snails, such as high humidity and adequate temperature and rainfall (LIMA et al., 2009; ALVES et al., 2011; MARTINS et al., 2012).

This study aimed to report on the spread of the parasite in the state of Minas Gerais and also provides the first report of naturally infected buffaloes in regions that had been considered to be unaffected.

Methods

Area of study

This study was conducted in the municipalities of São José da Lapa (latitude 19° 70' 57.2" south; longitude 43° 97' 47.6" west) and Pedro Leopoldo (19° 37' 12" south; 44° 2' 38" west), which are in the middle metropolitan region of Belo Horizonte, state of Minas Gerais, Brazil. These municipalities are lie within the basin of the Velhas river, and some of its tributaries, such as Córrego do Carranca, Ribeirão das Neves and Ribeirão da Mata, pass through several farms in the region, thus offering favorable epidemiological conditions for maintenance of the cycle of *Fasciola hepatica*.

Furthermore, the study area had a history of seven locally-reared adult buffaloes from which, after slaughtering, the livers were condemned due to infection by this parasite. Based on these data, fecal samples were collected from animals on the farm concerned and from neighboring farms, to search for other cases of infection.

Parasitological examinations of cattle and buffaloes

Nine farms drained by the streams Córrego do Carranca and Ribeirão da Mata were selected. On these farms, stool samples were collected directly from the rectum of each animal over the age of 12 months. The samples were collected in properly labeled plastic bags, packed in an insulated box and transported to the Veterinary Helminthology Laboratory, Federal University of Minas Gerais, where they were processed in accordance with the technique of Girão and Ueno (1982) in order to quantitatively and qualitatively diagnose *F. hepatica* eggs.

Molluscs

Searches for snails of the genus *Lymnaea*, an intermediate host for *F. hepatica*, were also conducted on these farms, on the banks of Córrego do Carranca and Ribeirão da Mata and on aquatic plants in these streams. Water tanks and drinking troughs used by the animals were also inspected when present. The specimens

found were transported to the laboratory and dissected under a stereoscopic microscope to investigate the presence of larval stages of the parasite. Among the molluscs collected, ten of them were separated out and kept in the laboratory for breeding. These specimens were kept in plastic vats containing dechlorinated water and fed with lettuce.

Viability of eggs coming from naturally infected cattle and buffaloes and susceptibility of L. columella collected on the farms studied, to infection by Fasciola hepatica

To evaluate the viability of the eggs of *F. hepatica* eliminated by naturally infected cattle and buffaloes, eggs recovered from these hosts were kept in petri dishes in an oven at 27 °C for incubation. After 13 days, the dish containing the eggs was placed under a spotlight for 1 hour, for the miracidia to hatch. Forty-eight molluscs of size 5 mm, which were obtained from the first generation of molluscs that had been collected on the farms, were used for this experimental infection. The specimens of *Lymnaea* were individually placed in the wells of a cell culture plate, each containing 2 ml of dechlorinated water and two miracidia. The snails were kept on the plates for a period of 24 hours. They were then transferred to plastic vats under the same conditions as described above. Starting 50 days after infection, the walls and bottom of the vat were inspected daily under a microscope, to search for cercariae and metacercariae eliminated by the snails.

Results and Discussion

A total of 250 stool samples was collected: 176 from cattle and 74 from buffaloes. The fecal examination showed that a total of 54 animals were positive for *Fasciola hepatica* eggs: 33 cattle (18.75% of the samples) and 21 buffaloes (28.37% of the samples). The number of *F. hepatica* eggs eliminated in four grams of feces, shown through analysis using the technique of Girão and Ueno (1982), ranged in the cattle from 1 to 84 eggs and in the buffalo from 2 to 330 eggs. The percentage of positive animals also varied between the farms studied, as shown in Table 1.

Several similar studies have been conducted describing buffaloes that were naturally infected with *F. hepatica*. Pile et al. (2001) made the first report on this parasite in young and adult buffaloes in the municipality of Marica, state of Rio de Janeiro, with a positivity rate of 2.5% among the 120 samples analyzed.

The highest prevalence rates found were recorded by Marques and Scroferneker (2003) in a study conducted in the state of Rio Grande do Sul. In an analysis on slaughtered animals, they found a prevalence of 10.34% among 377 livers from cattle and 20% among 105 livers from buffaloes infected with *F. hepatica*. Carneiro et al. (2010) reported natural infection by *F. hepatica* in buffaloes for the first time in the south of the state of Espírito Santo, with a positivity rate of 46.67% among 15 samples. These authors also reported that infected cattle and sheep were present on the farm, along with *L. columella*, but no examination of infection in snails was performed.

Table 1. Total number of fecal samples collected from cattle and buffalo on the farms studied, number of positive samples for *Fasciola hepatica* eggs and the percentage (%) positivity in each farm.

Farm	Species studied	Total number of samples collected	Positive samples for <i>F. hepatica</i> eggs	% positive animals in each farm
1	buffalo	19	19	100%
2	buffalo	26	1	3.84%
3	buffalo	29	1	3.44%
4	bovine	19	3	18.75%
5	bovine	37	5	13.51%
6	bovine	45	7	15.55%
7	bovine	44	6	13.63%
8	bovine	24	10	41.66%
9	bovine	7	2	28.57%

Lima et al. (2009) studied occurrences of *F. hepatica* in cattle in 120 municipalities in the state of Minas Gerais, from which a total of 6.255 fecal samples and 492 molluscs *Lymnaea* were collected. Among the municipalities studied, 16 showed positive animals. Among the molluscs collected, four were found to be naturally infected with *F. hepatica*, thus demonstrating the spread of fasciolosis in this state. Their data corroborate the present paper.

Alves et al. (2011) reported on the distribution of and factors connected with *Fasciola hepatica* infection in cattle in southern Espírito Santo and analyzed 50 farms selected in ten municipalities of the state. These authors observed a frequency of fasciolosis of 21.33% and emphasized that the area studied presented great epidemiological importance due to its geographical position covering two significant hydrographic basins. The rivers of these basins flow through the states of Espírito Santo, Minas Gerais and Rio de Janeiro, thus allowing the disease to spread not only in Espírito Santo but also in other states.

Bennema et al. (2014) reported on occurrences of *Fasciola hepatica* in cattle in Brazil and observed that in Minas Gerais all the infected municipalities were located in the south of the state. Our results showed the presence of the parasite in the center-south region of the state, again confirming the expansion of fasciolosis.

In relation to the intermediate host of *F. hepatica*, snails of the genus *Lymnaea* were only found on five of the nine farms investigated in this study. A total of 402 specimens were collected, of which two had immature forms of the parasite (0.5%). Other studies have been conducted to estimate the natural rate of infection of *Lymnaea* by *F. hepatica*, since the presence of the intermediate host in the environment is a fundamental factor in the epidemiology of fasciolosis. Coelho and Lima (2003) studied the natural infection rate of molluscs in the municipality of Itajubá, Minas Gerais, and found prevalences ranging from 0.9 to 5.2%. Their rates were higher than what was found in the present study (0.5%), but it is noteworthy that the work of Coelho and Lima (2003) was conducted in an area that is considered endemic for the parasite. Gomes et al. (2002) also examined naturally infected snails, and reported an infection rate of 5.22% among 134 specimens collected in the municipality of Goytacazes, in the state of Rio de Janeiro.

In our experimental infection of specimens of *Lymnaea*, cercariae of *F. hepatica* were found to be spontaneously eliminated from the 57th day post-infection onwards, thus demonstrating the viability of the eggs and the susceptibility of these snails to infection. These data corroborate other authors' observations (BORAY, 1996;

SOUZA et al., 2002), with spontaneous elimination of cercariae at close to 60 days post-infection.

The high prevalence found in buffaloes in the present study was because the farms had ponds and flooded areas with which the buffaloes had constant direct contact and where *Lymnaea* molluscs were present. It is important to emphasize all these factors, as well as the presence of infected native vertebrate hosts and environmental conditions favorable towards maintaining the cycle, as also reported by several authors (COELHO; LIMA, 2003; LIMA et al., 2009). This first reported case of naturally infected indigenous buffaloes and cattle in the state of Minas Gerais, in areas previously considered to be free from infection stands out in importance because it shows that this parasite has become dispersed to municipalities in this state that previously were considered to be unaffected.

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