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Ocorrência de *Fasciola hepatica* (Linnaeus, 1758) em capivara (*Hydrochoerus hydrochaeris*) (Linnaeus, 1766) em Minas Gerais, Brasil

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Abstract

Fasciola hepatica is a parasite that affects the hepatic ducts of several species of domestic and wild vertebrates, causing huge economic losses to livestock rearing worldwide. Reports on occurrences of *F. hepatica* in capybaras are an important epidemiological aspect of this disease, since these rodents can be a source of contamination for other animals and humans. In the present study, conducted in a rural area of the municipality of Confins, Minas Gerais, fresh feces from capybaras were collected from the ground near a lagoon at the edge of the Ribeirão da Mata river. These were examined using the technique of four metal sieves. *F. hepatica* eggs were recovered. This trematode species was confirmed by observing morphological characteristics and measuring the eggs recovered from the capybara feces, and through experimental infection of *Lymnaea columella* (Say, 1817) by miracidia from these eggs and subsequent infection of C57/BL06 mice with metacercariae originating from these infected mollusks. The data suggest the occurrence of natural cycle of *F. hepatica* in this region and provide a warning that expansion of the geographical distribution of this parasite by means of this rodent is possible. It is therefore important to adopting measures for epidemiological control of this helminthiasis.

Keywords: Fascioliasis, epidemiology, wildlife animals, rodents, *Hydrochoerus hydrochaeris*.

Resumo

Fasciola hepatica é um parasito que acomete os ductos hepáticos de diversas espécies de vertebrados domésticos e silvestres, causando grandes perdas econômicas na pecuária global. Relatos sobre a ocorrência de *F. hepatica* em capivaras constituem um importante aspecto epidemiológico dessa parasitose, visto que esses roedores podem ser fonte de contaminação para outros animais e humanos. No presente estudo realizado em área rural do município de Confins, Minas Gerais, foram coletadas no solo, próximo à lagoa marginal do rio Ribeirão da Mata, fezes frescas de capivaras. Essas foram examinadas pela técnica de quatro tamises metálicos. Foram recuperados ovos de *F. hepatica*. A confirmação da espécie desse trematódeo foi realizada por observação de características morfológica e mensuração dos ovos recuperados das capivaras, infecção experimental de moluscos *Lymnaea columella* (Say, 1817) por miracídeos oriundos desses ovos e pela infecção de camundongos C57/BL06 com metacercárias oriundas desses moluscos infectados. Os dados obtidos sugerem a ocorrência do ciclo natural de *F. hepatica* na região, alertando que a expansão da distribuição geográfica deste parasito por esse roedor é possível. Portanto, é importante a adoção de medidas para o controle epidemiológico desta helmintíase.

Palavras-chave: Fasciolose, epidemiologia, animais de vida selvagem, roedores, *Hydrochoerus hydrochaeris*.

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Introduction

Fasciola hepatica (Trematoda: Digenea) is a parasite in the family Fasciolidae that affects the liver ducts of sheep, cattle, buffaloes, goats, rodents and other mammals, and even humans. It is responsible for fasciolosis, which is a very important disease within veterinary and human medicine, given that it is a zoonosis.

In veterinary medicine, it constitutes a serious problem for domestic ruminant rearing and causes large losses and economic harm arising from condemnation of livers in slaughter houses, falls in milk, meat and wool productivity, growth retardation in young animals, decreased fertility, abortions, secondary bacterial infections, death of animals and high costs due to the medication that is used to control the parasitosis (DAEMON & SERRA-FREIRE, 1992; LIMA et al., 2009).

This disease has worldwide distribution, and in Brazil the largest enzootic area is located in the south, followed by the southeast and central-west. In the state of Minas Gerais, parasite records have arisen in isolation, and new foci have been reported, thus indicating the possibility of emergence of new areas of transmission and spreading of fasciolosis (LIMA et al., 2009; DRACZ & LIMA, 2014).

The economic and epidemiological importance of fasciolosis has led to greater numbers of studies directed towards ruminants of economic importance, such as cattle and sheep, or towards human infection. Nevertheless, there have been reports of infection by this disease in other animal species such as rodents, which suggests that they can possibly be considered to be reservoirs for the parasite cycle.

Capybaras (*Hydrochoerus hydrochaeris*) are the largest species of rodent found in Brazil and are also a definitive host of *F. hepatica* (SANTARÉM et al., 2006; EL-KOUBA et al., 2008). This rodent and its parasite present coincident distribution in lentic aquatic environments such as the backwaters of rivers, lakes, reservoirs and marshes. These environments are an essential factor in the development of the fluke cycle. Capybaras present adaptability to disturbed habitats, and this facilitates their survival in large urban centers and other regions subject to anthropic activity (FERRAZ et al., 2009). In addition to these factors, the defecation habits of capybaras in aquatic environments contribute towards maintaining the natural life cycle of the parasite. This makes it difficult to control this zoonosis, given that the mollusk genus *Lymnaea*, which is an intermediate host of *F. hepatica*, often occurs in these environments.

In some regions of the state of Minas Gerais, cattle and other animal species coexist with the presence of capybaras. Thus, it is important to study *F. hepatica* in capybaras, since these rodents can become infected and act as reservoirs, which would making it difficult to control the disease. The present work was carried out to study occurrences of *F. hepatica* in the population of capybaras in the municipality of Confins, Minas Gerais.

Materials and Methods

Study area

Stool samples were collected from capybaras living at a lagoon on the edge of the Ribeirão da Mata river, in the rural area of the municipality of Confins, which is located in the metropolitan region of Belo Horizonte, Minas Gerais (19° 40' 50.34" S, 43° 58' 23.42" W).

Collection and examination of feces

Eleven samples of fresh feces from capybaras were collected from the ground near a lagoon on the edge of the river, in the area of study, all on the same day in the morning. The feces were individually wrapped in labeled plastic bags, packed in an insulated box with ice and transported to the Veterinary Helminthology Laboratory of the Institute of Biological Sciences, Federal University of Minas Gerais.

In order to recover *F. hepatica* eggs, the feces were processed and analyzed in accordance with the technique described by Girão & Ueno (1985). The eggs found were counted and measured under a microscope.

Viability of eggs, miracidia and metacercariae

The eggs that were found through examining the feces were transferred to Petri plate containing dechlorinated water, with the aid of a Pasteur pipette. The plate was kept in an incubator at 27 °C. From the seventh day onwards, the plates were examined daily under a stereoscopic microscope at 40x magnification, in order to follow the formation and development of miracidia. Plates containing eggs with developed miracidia were placed under a spotlight (60 W) at a distance of 50 cm for a period of one hour, to stimulate hatching.

The miracidia that hatched were used to infect *L. columella* mollusks that had been reared in our laboratory. Infection was performed using 24-well culture plates (Kartel SPA). The mollusks were placed individually into wells containing 2 mL of dechlorinated water and three miracidia were added using a Pasteur pipette. The plates were kept under a focused light of 60 W at a distance of 50 cm for a period of 24 hours. After infection, the mollusks were kept in plastic tubs with dechlorinated water and were fed with lettuce. On the 50th day after infection, the mollusks were removed from the tubs and were placed in individual Petri plates lined with plastic film (Parafilm® Laboratory Film, American National CanTM) in order to facilitate recovery and encystment of the metacercariae. The plates were exposed to a focused light of 60 W at a distance of 50 cm for 5 hours a day, to stimulate elimination of cercariae and formation of metacercariae.

The viability of the metacercariae was evaluated through experimental infection of seven female mice (C57/BL06) that were eight weeks old. Each mouse was infected orally with three metacercariae. These were suspended in dechlorinated water and administered with the aid of a syringe, by means of needle gavage.

The procedure for experimental infection of the mice had previously been submitted for approval to the Ethics Committee for

Use of Animals (CEUA) of the Federal University of Minas Gerais. It was approved under the protocol number CEUA181/2015.

Starting on the 30th day after infection, feces were collected from the mice every day and were processed by means of the Girão & Ueno technique (1985) for quantitative and qualitative diagnosing of *F. hepatica* eggs.

Results and Discussion

Through examination of the 11 capybara feces samples in the laboratory, one sample (9.1%) yielded an average of 16 eggs of *F. hepatica* per gram of feces examined. From the eggs recovered, 20 were separated and measured under a microscope. These presented an average length of 142.74 µm (SD ± 7.63) and width of 75.74 µm (SD ± 7.83), as shown in Table 1.

Regarding the viability of the eggs recovered from capybara feces, formation and hatching of active miracidia from all eggs was observed after incubation. These miracidia were used to experimentally infect 50 *L. columella* mollusks.

Spontaneous elimination of cercariae was observed starting on the 57th day after infection of the mollusks. These encysted on the plastic film that covered the inner surface of the Petri plate. A total of 2,403 metacercariae were obtained from the 13 surviving mollusks (26%).

These metacercariae obtained were used to experimentally infect mice. On the 77th day after infection, 60% of the mice were still alive and eggs were observed in their feces. Egg elimination

continued to be observed until three months after infection, at which time the mice were necropsied and adult parasites were recovered from the bile ducts and gallbladder.

This confirmation of the viability of the eggs recovered from the capybaras and of the cycles in *Lymnaea* and in mice demonstrates that capybaras are definitive hosts for *F. hepatica*. This suggests that capybaras have real importance in maintaining the wild cycle. It is important to remember that capybaras disperse into different groups, to take possession of territory and search for food, while always moving near water courses, and that they can act as disseminators of fasciolosis. On the farm where the present study was conducted, capybaras were living in the same area as cattle and humans, all with free access to the lagoon where the feces sampling was carried out. The mollusc *L. columella* is also present at this location, thus facilitating encounters between the miracidia that hatched from the eggs and the intermediate host and leading to cattle infection.

The average size of the *F. hepatica* eggs observed in this study was similar to what was described by El-Kouba et al. (2008), who reported that the dimensions of eggs obtained from capybaras were 138.56 µm in length and 72.96 µm in width. In the present study, it was also observed that the average number of *F. hepatica* eggs released per gram of feces from the capybaras was lower than the average number of eggs released by cattle and buffaloes present in municipalities neighboring the area of this study, as reported by Dracz & Lima (2014). The capybara population is increasing, and therefore these rodents can participate in transmission and maintenance of the *F. hepatica* cycle in the study area.

Santarém et al. (2006) reported occurrences of adult parasites in the liver of an adult capybara that was found dead in a park in the city of Presidente Prudente, state of Sao Paulo, Brazil. The parasite was identified as *F. hepatica* and, during the autopsy, liver lesions characteristic of infection by this parasite were observed. However, no eggs were recovered from the feces.

El-Kouba et al. (2008) studied the presence of *F. hepatica* eggs in capybaras in three parks in Curitiba, state of Paraná - Brazil, and found that the prevalence ranged from 0 to 100% in samples collected from the populations living in these parks.

Bellato et al. (2009) studied occurrences of this parasite in populations of cattle and capybaras on 19 farms in the municipality of Timbó, Santa Catarina - Brazil. They reported occurrences of 18.12% in cattle and 8.96% in capybaras that were naturally infected by *F. hepatica*. They pointed out that the difference in these results could be explained by the diversity of environmental conditions, management and surveying at the sites where the studies were conducted.

The population of capybaras in Minas Gerais has shown significant growth over recent years. Advancement of farming into areas close to wildlife habitats provides contact between human populations, domestic animals and wild animals, and it facilitates maintenance of this disease between hosts and environments and thus establishment of new parasite-host relationships (EL-KOUBA et al., 2008).

This is the first report on capybara naturally infected by *F. hepatica* in the state of Minas Gerais. It emphasizes the importance of epidemiological studies in this region, with the aim of searching for new foci of fasciolosis. Such studies may

Table 1. Mean and standard deviation of length and width measurements (µm) on *Fasciola hepatica* eggs recovered from the feces of a naturally infected capybara (*Hydrochoerus hydrochaeris*), in the municipality of Confins, Minas Gerais.

<i>Fasciola hepatica</i> eggs	
Length (µm)	Width (µm)
130.94	80.23
142.46	70.32
142.98	73.38
135.70	71.66
138.94	76.02
146.89	66.13
147.62	66.55
137.82	75.24
149.68	81.46
156.64	91.03
143.54	79.12
139.41	75.12
146.73	81.36
139.12	69.45
130.49	91.74
144.64	85.02
146.47	78.41
151.36	67.04
154.15	70.65
129.21	65.04
142.74 ± 7.63	75.74 ± 7.83

contribute towards adoption of control measures that are more comprehensive, with the aim not only to treat cattle and other domestic ruminants, but also to treat and implement control measures among wildlife that can act as reservoirs for this emerging zoonosis in this state.

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