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CASE REPORT

GRANULOMATOUS HEPATITIS CAUSED BY *Calodium hepaticum* IN A CAPTIVE MANDRILL (*Mandrillus sphinx*) IN BRAZIL

Sarah Raquel Jesus Santos Simões[1], Amanda Garcia Pereira[1], Maitê Cardoso Coelho da Silva[1], Leticia Colovatti Mariano[1], Fernanda Lefort[1], Camilla de Souza Amorim[1], Márcio Junio Lima Siconelli[2], Livia de Oliveira Andrade[1], Estevam G. Lux Hoppe[1] and Karin Werther[1]

ABSTRACT

The parasite *Calodium hepaticum* is a zoonotic, cosmopolitan nematode often associated with rodents. These nematodes have low host specificity and zoonotic potential, affecting the liver of their hosts. The objective of this work is to describe the first case, as far as the authors are aware, of *C. hepaticum* causing granulomatous hepatitis in captive *Mandrillus sphinx*. A necroscopic examination was performed and the organs were removed and evaluated; then, 1 cm^3 fragments were collected and preserved in 10% buffered formalin. After fixation, the samples were submitted to histotechnical processing to make histological slides. At macroscopic examination, the liver showed heterogeneous red surface with light brown and whitish areas distributed throughout the parenchyma, with diverse punctiform structures measuring 1 to 3 mm in diameter. Histopathology revealed abundant multifocal granulomas with fibrosis and bioperculated barrel-shaped eggs, typical of *C. hepaticum*. Artificial tissue digestion of the liver was performed and the morphometric measurements of the parasite were as follows: 0.054 ± 0.002 mm in length, 0.031 ± 0.008 mm in width, wall thickness of 0.004 ± 0.0007 mm and opercula of 0.006 ± 0.001 mm. Parasitism was not the cause of death, but an incidental finding. However, further investigations are essential to choose control and prevention measures to prevent the infection of other animal groups and even humans.

KEY WORDS: Nematoda; parasitism; primates; zoo medicine; wildlife pathology.

1. São Paulo State University, School of Agricultural and Veterinarian Sciences, Jaboticabal Campus, Jaboticabal, São Paulo State, Brazil.
2. Municipal Secretary of the Environment, Bosque e Zoológico Municipal Dr. Fábio de Sá Barreto, Ribeirão Preto, São Paulo State, Brazil.

Corresponding author: Sarah Raquel Jesus Santos Simões. E-mail: sarahraqueljesusss@gmail.com

Sarah Raquel Jesus Santos Simões ORCID: https://orcid.org/0009-0000-2289-8315; Amanda Garcia Pereira ORCID: https://orcid.org/0000-0002-0816-2798; Maitê Cardoso Coelho da Silva ORCID: https://orcid.org/0000-0002-0047-3911; Leticia Colovatti Mariano ORCID: https://orcid.org/0000-0001-7273-8061; Fernanda Lefort ORCID: https://orcid.org/0009-0009-3125-7232; Camilla de Souza Amorim ORCID: https://orcid.org/0009-0000-1781-0876; Márcio Junio Lima Siconelli ORCID: https://orcid.org/0000-0001-5340-0962; Livia de Oliveira Andrade ORCID: https://orcid.org/0000-0001-8820-2624; Estevam G. Lux Hoppe ORCID: https://orcid.org/0000-0003-3958-7227; Karin Werther ORCID: https://orcid.org/0000-0002-8822-488X

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INTRODUCTION

Calodium hepaticum (Bancroft, 1893) (Nematoda: Capillaridae) is a cosmopolitan zoonotic nematode with low host specificity, which causes hepatic lesions in their hosts. This parasite has been reported to affect more than 180 species of mammals, including humans. The main reservoirs are murine rodents, especially the synanthropic species. Since the diagnosis of this parasitic infection depends on the histopathological examination of the liver, the disease is not often reported (Fuehrer, 2014 a, b).

Adult *C. hepaticum* are long, slender nematodes with tapering anterior ending, in correspondence to the stichosome and a thicker posterior part of the body. They present sexual dimorphism and the eggs are barrel-shaped, with well-developed polar plugs and striated shell, measuring from 40 to 67 × 27 to 35 µm (Schmidt, 2001; Fuehrer, 2014b).

There are several reports of hepatic capillariosis in non-human primates and these may vary from no reaction to the parasite to acute granulomatous hepatitis (Lowenstine et al., 2018). The ingested eggs hatch in the cecum region, where the larvae penetrate the mucosa and reach the liver through the portal venous system. In the liver, they become adults and produce eggs until they die. The main route of transmission is the predation of infected animals, but it can also occur through carcass decomposition and consequent contamination of the soil (Anderson, 2000; Moreira et al., 2013).

The objective of this case report is to describe the first case, as far as the authors are aware, of *C. hepaticum* causing granulomatous hepatitis in a captive *Mandrillus sphinx* (Primates: Cercopithecidae) maintained at a zoo in the northwest region of the State of São Paulo, Brazil.

MATERIAL AND METHODS

A male mandrill (*M. sphinx*) from a Brazilian zoo, 30 years old, weighing 21.5 kg, died from complications from bone exposure due to a support eschar in the sacral region. The animal's enclosure was of the cage type, with a floor partially made of cement and grass with soil. The walls were in some parts made of masonry and in others with mesh. On top there was a roof made of tiles to protect against rain and sun.

The necroscopic examination was performed through a mento-pubic incision after the skin was removed and the abdominal cavity was exposed. The position of the organs was evaluated, and a negative thoracic pressure test was performed. The ribs were sectioned in the articular region and the thoracic cavity was evaluated for the position of the organs. The organs were removed, evaluated, photographed, and collected in 1 cm^3 fragments in 10% buffered formalin for 24 to 48 hours. After being fixed, the samples were dehydrated in increasing alcohol solutions, cleared in xylol, and embedded in paraffin (Ribeiro et al., 2013). To make the histological slides, the paraffin blocks passed through a rotating microtome, which sectioned 3 µm thick samples. The sections were placed on glass slides, deparaffinized, and stained with hematoxylin and eosin, in addition to stained to Ziehl-Neelsen staining, for identification of Acid-Alcoholic Resistant Bacilli (AARB), and Gram staining for other bacteria.

The artificial digestion technique was based on the magnetic stirrer method by the European regulation EC 2075/2005, which is used for infection surveillance by *Trichinella* in wild animals, in a way adapted by the amount of sample. Two grams of liver sample from the infected animal was digested in 40 mL of digestive liquid (0.3 g of pepsin and 0.4 ml of hydrochloric acid [HCl]). Digestion was stirred for 15 minutes at 44-46 ºC in a 100 mL glass beaker using a magnetic stirrer with a heated plate. After digestion, the fluid was sieved through a mesh sieve (180 µm) and placed in the separation funnel, leaving it to rest for 20 minutes. Due to the small amount of liquid and sample, dilution was not performed. Using a Pasteur pipette, one to two drops of the sediment were collected and placed on a slide microscope glass, covered with a coverslip, and visualized under an optical microscope (Olympus BX-51), equipped with Olympus BX-51 QColor3 digital camera (Olympus America Inc., Center Valley, PA, USA), at 10× magnification.

RESULTS

During a necroscopic examination, hepatomegaly was noticed. The liver was heterogeneous red with light brown areas and whitish points, firm, from 1 to 3 mm distributed throughout the parenchyma (Figure 1). In addition, the organ drained blood when cut and the gallbladder was full, green in color, with the presence of biliary sludge and lumps. Histopathology showed abundant multifocal granulomas with fibrosis, cellular debris, and elliptical parasite eggs, with two opercula and eosinophilic heterogeneous material inside, suggestive of *C. hepaticum* eggs (Figures 2 and 3). Furthermore, the liver showed moderate to severe multifocal perivascular hemosiderin, moderate multifocal sinusoid congestion, moderate to severe multifocal perivascular predominantly lymphocytic inflammatory infiltrate, moderate hepatocyte degeneration with loss of cell delimitation, and diffuse disarrangement. The samples collected from the liver were stained using Ziehl-Neelsen, for identification of Acid-Alcoholic Resistant Bacilli (AARB); and Gram staining for other bacteria identification, however all samples tested were negative.

In artificial digestion, 10 eggs with double striated shells and two operculated plugs were identified, belonging to the whipworm nematode *C. hepaticum*. Morphometric measurements were constituted of mean and standard deviation. These are 0.054 ± 0.002 mm long, 0.031 ± 0.008 mm wide, wall thickness 0.004 ± 0.0007 mm and opercula 0.006 ± 0.001 mm (Figure 4).

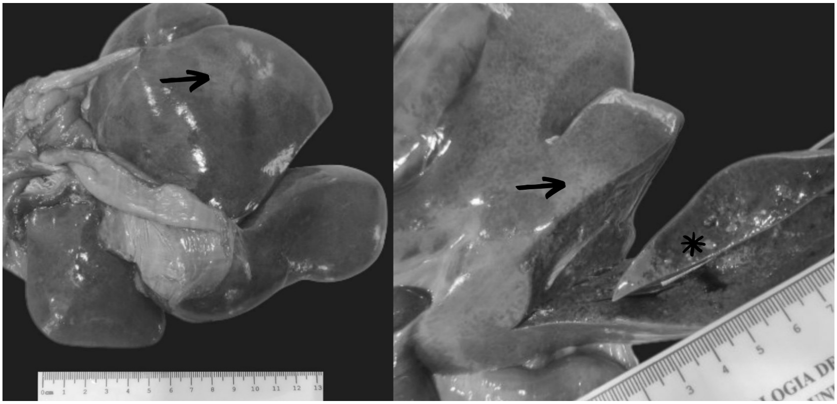

Figure 1. Mandrillus sphinx liver with hepatomegaly, multifocal pale spots (arrows), and with presence of whitish punctiform structures, with firm consistency, measuring from 1 to 3 mm in the parenchyma (asterisk).

Figure 2. Photomicrograph of *Mandrillus sphinx* liver with serpiginous lesions (arrows) caused by the *Calodium hepaticum*, HE.

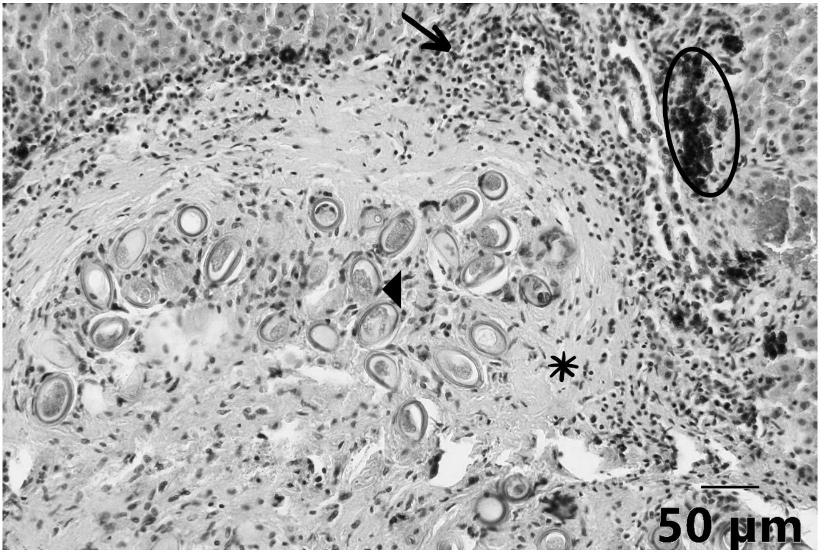

Figure 3. Photomicrograph of *Mandrillus sphinx* liver with parasitic granuloma formed by abundant fibrous tissue (asterisk), *Calodium hepaticum* eggs (arrowhead), inflammatory infiltrate (arrow), and hemosiderin (circle), HE.

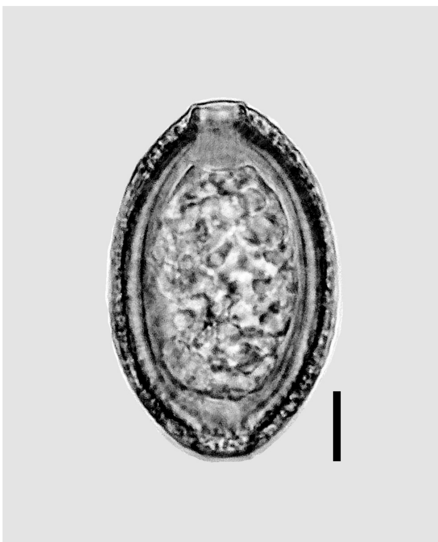

Figure 4. Photograph of *Calodium hepaticum* egg after artificial digestion technique. Scale: 10 µm.

DISCUSSION

There are reports of micronodules in the liver on macroscopy, in addition to observing in histopathology: hepatic necrosis, inflammatory reaction, fibrosis, and parasite eggs (Palma et al., 2009). Furthermore, in severe granulomatous inflammation, adult females cause serpiginous pathways when depositing eggs in the liver (Lowenstine et al., 2018). These findings are compatible with those found in the mandrill and corroborate the conclusion of the diagnosis.

Zoo animals exposed to contact with wild rodents are at greater risk of becoming infected with *C. hepaticum* (Redrobe & Patterson-Kane, 2005; Pizzi et al., 2008). In a zoo in the United States, 75% of captured rats were infected with *C. hepaticum*, increasing the probability of animals and humans contracting the disease (Redrobe & Patterson-Kane, 2005). In this context, the control of rodents in zoos should be a priority to reduce the parasite load and prevent other diseases that are carried or transmitted by these animals.

There are several reports of *C. hepaticum* infection in non-human primates. Some reported species are *Ateles paniscus, Gorilla gorilla beringei, Pithecia Pithecia, Macaca nigra, Saguinus bicolor* and *Callithrix geoffroyi*. These authors describe injuries similar to those presented in our findings, with fatal injuries reported only in infants of mountain gorillas (Graczyk et al., 1999; Pizzi et al., 2008; Stidworthy et al., 2009; Soares et al., 2011; Lowenstine et al., 2018). In the case of the mandrill, parasitism was also not the cause of death, but an incidental finding. Although there are areas of fibrosis and intense inflammation in the liver tissue, they occupied a small part of the organ, which did not compromise its proper functioning. The animal in question presented several other injuries that led to death, the main one being bone exposure in the sacral region with contamination and purulent secretion, leading to a septic condition. This is the first report of *C. hepaticum* in mandrill, which confirms the susceptibility of this species to this parasite.

In humans, the prevalence of parasitosis is related to the health status of the population, as well as direct contact with domestic and wild animals (Soares et al., 2011). Hepatic capillariasis is a neglected parasitic disease that causes liver pathology. Different manifestations range from mild to severe and can lead to death (Wang et al., 2019). Liver changes include destruction of the parenchyma, eosinophilic granulomas, and fibrosis (Barbosa & Andrade, 2010; Gaban et al., 2010) and the diagnosis is still a challenge due to the biological characteristics of the parasite (Li et al., 2010).

Although injuries that compromise liver function are rare, it is important to investigate the parasitosis and to report it, so that it is possible to invest in control and prevention measures. Since the animal is in a zoo, it can lead to infection in other more susceptible animal groups and even in humans.

Additionally, it is essential to control rodents in captivity since they are the main reservoirs of this parasite.

CONFLICT OF INTEREST

The authors report there are no competing interests to declare.

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