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Calodium hepaticum (Nematoda: Capillariidae) in synanthropic rodents (*Rattus norvegicus* and *Rattus rattus*) in Eastern Amazonia

Primeiro relato de *Calodium hepaticum* (Nematoda:Capillariidae) em roedores sinantrópicos (*Rattus norvegicus* e *Rattus rattus*) na Amazonia Oriental

Vera Lúcia Coimbra Moreira[1]; Elane Guerreiro Giese[2]; Djane Clarys Baía da Silva[1]; Francisco Tiago de Vasconcelos Melo[1]; Adriano Penha Furtado[1]; Arnaldo Maldonado Jr[3]; Jeannie Nascimento dos Santos[1]*

[1]Laboratório de Biologia Celular e Helmintologia "Profa. Dra. Reinalda Marisa Lanfredi", Instituto de Ciências Biológicas, Universidade Federal do Pará – UFPA, Belém, PA, Brasil

[2]Laboratório de Histologia e Embriologia Animal, Instituto de Saúde e Produção Animal, Universidade Federal Rural da Amazônia – UFRA, Belém, PA, Brasil

[3]Instituto Oswaldo Cruz – IOC, Fundação Oswaldo Cruz – Fiocruz, Rio de Janeiro, RJ, Brasil

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Abstract

Calodium hepaticum (syn. *Capillaria hepatica*) is a trichurid nematode that parasitizes the hepatic parenchyma of rodents and other mammals. Infections in humans are rare, although they have been reported worldwide. A number of factors contribute to the distribution of this zoonosis, particularly the presence of dense populations of rodents associated with relatively poor urban environments, such as those found in parts of the northern Brazilian city of Belém in the eastern Amazon Basin. This study quantified *Calodium* infections in commensal synanthropic rodents in Belém. Rodents were captured in three neighborhoods characterized by poor public sanitation and the city's highest incidence of human leptospirosis. A total of 50 rodents were captured (26 *Rattus rattus* and 24 *R. norvegicus*), and 23 (10 *R. rattus* and 13 *R. norvegicus*) presented macroscopic lesions typical of *C. hepaticum*. Light microscopy of fresh samples and histological specimens permitted the identification of larvae and adult specimens containing numerous eggs with a double-striated shell and bipolar opercula with plugs. This is the first report of *C. hepaticum* in *R. rattus* and *R. norvegicus* from the Amazon Basin, and it shows a considerable risk of transmission to the local human population.

Keywords: *Calodium hepaticum*, *Rattus rattus, Rattus norvegicus*, public health, synanthropic animals, zoonosis.

Resumo

Calodium hepaticum (syn. *Capillaria hepatica*) é um nematódeo trichurídeo parasito de parênquima hepático de roedores e outros mamíferos. As infecções em humanos são raras, mas são relatadas em diversas regiões do mundo. Numerosos fatores contribuem para a distribuição desta zoonose, particularmente, uma densa população de roedores associada com ambientes urbanos com carência de saneamento básico, tais como aqueles encontrados em algumas cidades da região Norte do Brasil, como a cidade de Belém, localizada na Amazônia Oriental. Este estudo quantifica e demonstra a infecção por *Calodium* em roedores comensais sinantrópicos, de três bairros da cidade de Belém, Estado do Pará, com carência de saneamento público e alta incidência de leptospirose humana. Um total de 50 roedores foram capturados para análise (26 *Rattus rattus* e 24 *R. norvegicus*) e destes, 23 (10 *R. rattus* e 13 *R. norvegicus*) apresentaram típicas lesões hepáticas, macroscópicas, causadas por *C. hepaticum*. A análise de amostras por microscopia de luz direta e histopatológica do fígado dos roedores permitiu a identificação de espécimes desse parasito em fase larvar e adulta, além de numerosos ovos apresentando dupla casca estriada e dois tampões operculares. Este é o primeiro registro da ocorrência de *C. hepaticum* in *R. rattus* and *R. norvegicus* na região Amazônica, alertando para um considerável risco de transmissão para a população humana.

Palavras-chave: *Calodium hepaticum*, *Rattus rattus, Rattus norvegicus*, saúde pública, animais sinantrópicos, zoonose.

***Corresponding author:** Jeannie Nascimento dos Santos
Laboratório de Biologia Celular e Helmintologia,
Instituto de Ciências Biológicas, Universidade Federal do Pará – UFPA,
Av. Augusto Correia, Guamá, CEP 66075-110, Belém, PA, Brasil
e-mail: jeannie@ufpa.br

Introduction

Calodium hepaticum is a zoonotic parasite of the hepatic parenchyma of mammals, primarily rodents (GALVÃO, 1981; LI et al., 2010). The nomenclature of this species (Trichuroidea: Capillariidae) [syn. *Capillaria hepatica* (Bancroft 1893)] was modified based on the taxonomic revision of the nematodes of the family Capillariidae (MORAVEC, 1982).

The life cycle of this parasite is monoxenic (KATARANOVSKI et al., 2010). In the hepatic parenchyma, eggs are metabolically active but not infective. They become infective in the environment after 28 days (GALVÃO, 1981). Infective eggs are ingested orally, and the larvae hatch in the region of the caecum, penetrate the mucous membrane, and reach the liver through the portal venous system, in which they develop into adults after molting four times (LI et al., 2010). They remain in the liver and produce eggs until their death (GALVÃO, 1981; RESENDES et al., 2009). Cannibalism, which is common in rodents, is considered to be the principal mechanism of egg dispersal, which is essential to the life cycle of this nematode (PIAZZA et al., 1963; NABI et al., 2007), but cannibalism is not the only method of dispersal.

Humans become infected by ingesting food or water contaminated with eggs of *C. hepaticum*. Children also become infected through the ingestion of soil (FUEHRER et al., 2011). The manifestation is usually subclinical. Symptomatic cases present chronic fever and intense eosinophilia, in which the eosinophil count may exceed normal levels by as much as 85% (GALVÃO, 1981). The presentations associated with hepatomegaly constitute the classical triad of human capilariasis. Hepatic fibrosis is common, and more serious cases can be fatal (SAWAMURA et al., 1999, FUEHRER et al., 2011).

The diagnosis of *C. hepaticum* infections is based on the detection of adult and juvenile worms and the typical egg, which has bipolar opercula with plugs (REPERANT; DEPLAZES, 2005; RESENDES et al., 2009; LI et al., 2010).

Although human cases of capillariasis are rare, subclinical infections could occur because of the difficulty of diagnosis (GALVÃO, 1981; SAWAMURA et al., 1999; RESENDES et al., 2009), and reports have been published in the Americas, Europe, Asia, Africa, and Oceania.

In Brazil, *C. hepaticum* has been recorded in state of São Paulo in 43% of rodents captured by Piazza et al. (1963); in the state of Bahia, where it was found in rodents (*R. rattus, R. norvegicus* and *Mus musculus*) with a prevalence of 64% and in children (GALVÃO, 1979, 1981); and in Rondônia, in a human population inhabiting a swampy riverside area (CAMARGO et al., 2010).

Belém is a typical city of Amazonia marked by the presence of heavy rainy seasons and small rivers that propitiate inundations, primarily in poorly neighborhoods with low urban planning and sanitation, factors that facilitate the proliferation of rodents, diseases and infectious agents transmitted by these animals. This situation is complicated by the high levels of human leptospirosis in the city (IBGE, 2010). Thus, the present work aimed to determine the presence of *C. hepaticum* in commensal synanthropic rodents in the neighborhoods of the city.

Materials and Methods

Study area

The city of Belém is located in the Amazon Basin (01° 27' 20" S and 48° 30' 15" W), with a hot and humid climate typical of tropical rainforests. The temperatures range between 25 °C and 26 °C, and the mean annual precipitation is 2,834 mm (BELÉM, 2010).

The three neighborhoods selected for the present study – Guamá, Montese, and Jurunas (Figure 1) – were chosen because they present the highest rates of cases of human leptospirosis according to the Disease Information and Notification System of the Brazilian Government (SINAN). These three neighborhoods are characterized by a lack of infrastructure, unregulated urban development, and a generally low-income population. Many of the households are built in areas prone to flooding or are stilt buildings constructed over bodies of water, normally with inadequate sanitation (BELÉM, 2010).

Specimen collected

Rats were collected during four field campaigns between January 2009 and August 2010. A total of 60 Tomahawk live traps (Tomahawk Live Trap Company, Tomahawk, WI, USA) were set

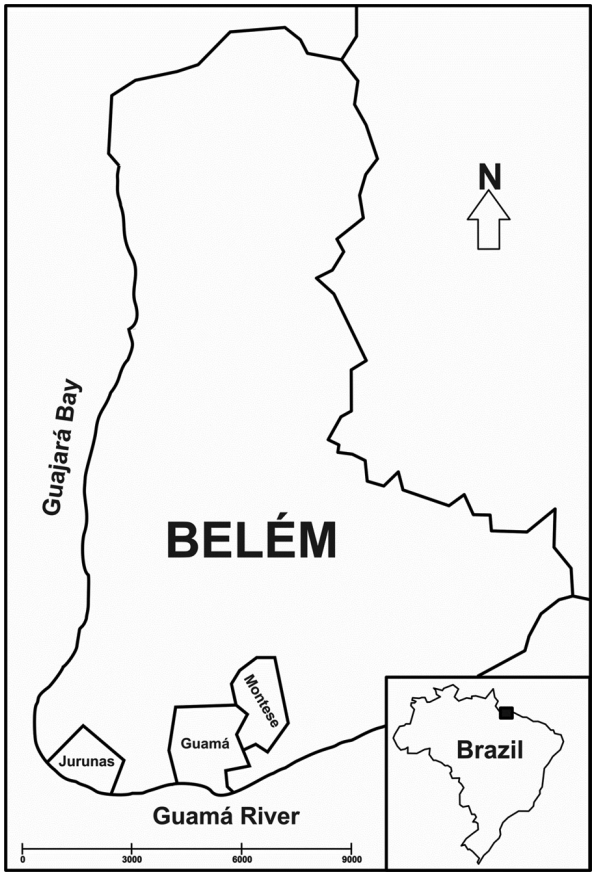

Figure 1. Geographic localization of neighborhoods in Belém, capital of Para State. Insert: Map of Brazil that indicates the location of the city (01° 27' 20" S and 48° 30' 15" W). (Scale bar = km)

along 30 m transects located primarily within residential areas in the three study neighborhoods. The traps in each transect were baited on four consecutive nights with rodent pellets and fresh fruit, such as mango and banana.

Urban rodents are synanthropic animals that are considered to pose a risk to public health. Capture is enforced by municipal law n. 8498/2006, and the methods of euthanasia and necropsy followed resolutions 722/2002 and 714/2002 of the Veterinary Medicine Ethics Code. Immediately upon capture, the rodents were taken to the UFPA campus, where they were weighed, sedated with ketamine chloride (100 mg/kg) applied intramuscularly, measured, euthanized by total bleeding through cardiac puncture, and necropsied.

Laboratory examinations: macroscopic, stereomicroscopic microscopic and histological examinations

Fresh liver specimens with macroscopic lesions were analyzed in phosphate-buffer under light microscopy and stereomicroscopy. Samples were fixed in Davidson fixing solution (acetic acid, 95% ethanol, 37% formaldehyde, and water at a proportion of 1:3:2:4) and processed for embedding in paraffin or mounting in HistoResin Mounting Media (Leica Microsystems, Heildelberg, Germany) for histological analysis. Sections of 2-3 µm of the samples set in HistoResin were stained with 1% toluidine blue and Gomori trichrome, and samples embedded in paraffin were stained with hematoxylin and eosin. The samples were analyzed under an Olympus BX 41 microscope and photographed with an Olympus DP72 camera.

Results

In total, 50 rats were captured: 26 *R. rattus* (12 males and 14 females) and 24 *R. norvegicus* (14 males and 10 females). The specimens were identified based on their phenotypic characteristics.

Macroscopic and stereomicroscopic examinations:

Twenty-three (42%) rates presented diffuse macroscopic lesions in the liver: 10 *R. rattus* (4 males and 6 females) and 13 *R. norvegicus* (7 males and 6 females). These lesions were characterized by extensive, sinuous, and irregular blemishes on the surface of the hepatic parenchyma, with a cheese-like appearance and white-yellowish coloration (Figure 2A). The observation of these lesions under a stereomicroscope permitted the visualization of foreign bodies in the tissue of the organ. The bodies could be removed, albeit in fragments (Figure 2B).

Microscopic examinations

Microscopic analysis identified these fragments as segments of nematodes enveloped in a fine cuticle, which contained numerous eggs with a transversally striated double casing and bipolar opercula (Figure 2C).

Histological examinations

Histological sections of the parasitized livers revealed details of the helminth at different stages of maturation, including adults, which were identified by the presence of large numbers

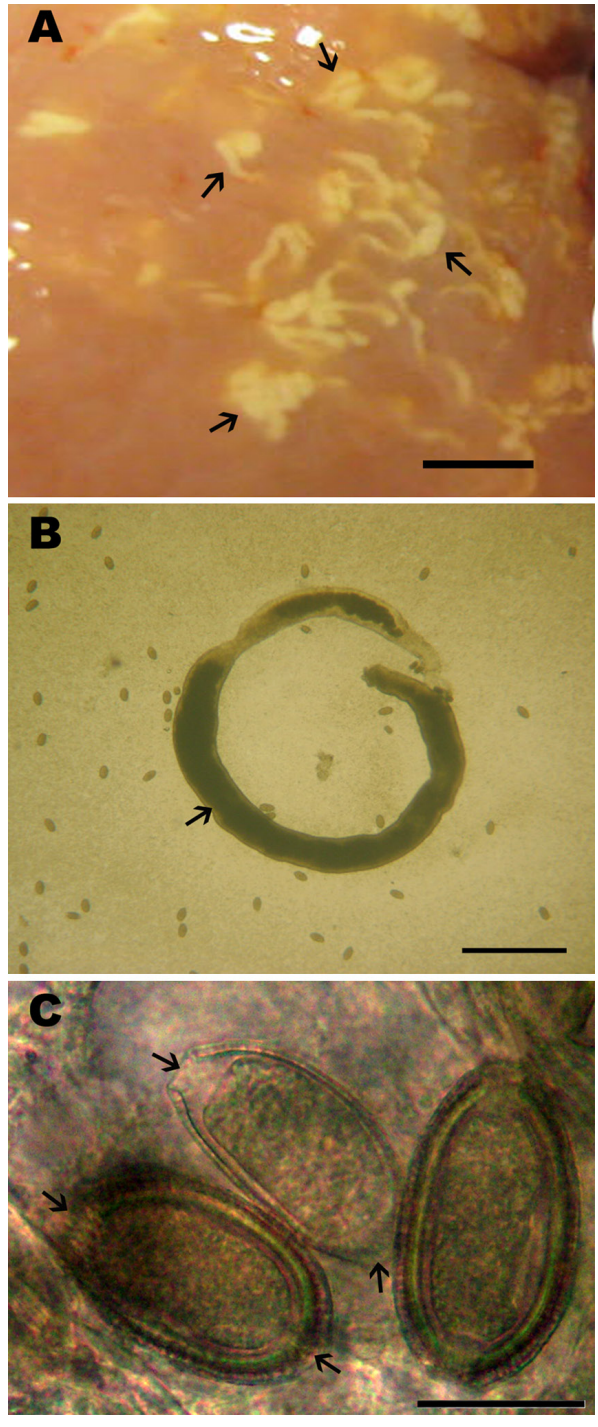

Figure 2. Characteristics of *Calodium hepaticum* infection in the liver of *Rattus rattus* and *R. norvegicus.* A) Macroscopic view of the liver showing irregular and sinuous pale blemishes (arrow) with a cheese-like appearance (Scale bar = 2 mm). B) Partial female *C. hepaticum* specimen with a uterus full of eggs (arrow) (Scale bar = 360 µm). C) Elliptical eggs enveloped in a striated double casing with bipolar opercula with plugs (arrow) (Scale bar = 20 µm).

of eggs in their interior (Figures 3A-B), as well as larval stages (Figure 3A-insert), which were smaller and thinner than the adults. Numerous eggs with characteristics of *C. hepaticum* were found in the hepatic parenchyma (Figure 3C). Because of the large numbers of parasites, the lesions in the tissue of the hepatic parenchyma were extensive, resulting in considerable mechanical pressure, which resulted in severe alterations of the structure of the lobes of the liver and the distribution of the arterial and venous vessels and biliary ducts and alterations in the distribution of hepatocytes around the centrolobular veins (Figures 3A-B).

Deposits of conjunctive tissue were observed around each parasite (Figure 3B) in addition to a buildup of infiltrated leukocytes and deposits of hemosiderin resulting from the decomposition of hemoglobin seeping from the blood vessels (Figures 3A-B). Septal fibrosis was also found, suggesting the chronic nature of the infection (Figure 3A).

Discussion

The watercourses that are distributed throughout the city contribute to a beautiful background in Belém, but the absence of adequate urban planning and sanitation propitiates flooding in some areas, garbage accumulation and, subsequently, synanthropic animals. The presence of rodents in urban areas is common because they have access to habitations and food stores. In Belém, one of the consequences of the coexistence of rodents and humans is the high prevalence of human cases of leptospirosis. Surveys on the risks of this co-habitation are necessary to identify a possible zoonosis to which the population could be exposed.

Those factors necessitate research on potential zoonotic helminthes from commensal rodents (*Rattus* spp.), which have been described as a primary reservoir of *C. hepaticum* (BARRETT; GORDON, 1972; LI et al., 2010). In this work, rodents from three different neighborhoods in Belém, which has the highest reported numbers of human leptospirosis cases and a high number of rodents in habitations, were captured.

The macroscopic aspects of the lesions observed in the livers of the infected rodents captured in the present study corresponded to the description of *C. hepaticum* reported by Barrett and Gordon (1972) and Ceruti et al. (2001). This parasitism was confirmed using light microscopy and visualization of the helminths in different stages of development, supporting the diagnosis of infection by *C. hepaticum*, similar to studies of Barrett and Gordon (1972), Farhang-Azad and Schlitter (1978), Reperant and Deplazes (2005) and Resendes et al. (2009).

This is the first report of *C. hepaticum* in *R. rattus* and *R. norvegicus* from the Amazon region within the urban area of Brazil and indicates a considerable risk of transmission to the local human population.

In Brazil, leptospirosis and bubonic plague are the only rodent-borne diseases included in the compulsory notification lists of the government's SINAN record system (BRASIL, 2005). The present study appears to support the conclusion that the occurrence of human leptospirosis in a given area is a clear indicator of the potential for the transmission of other rodent-borne diseases, such as capillariasis, a zoonosis that is poorly known by health

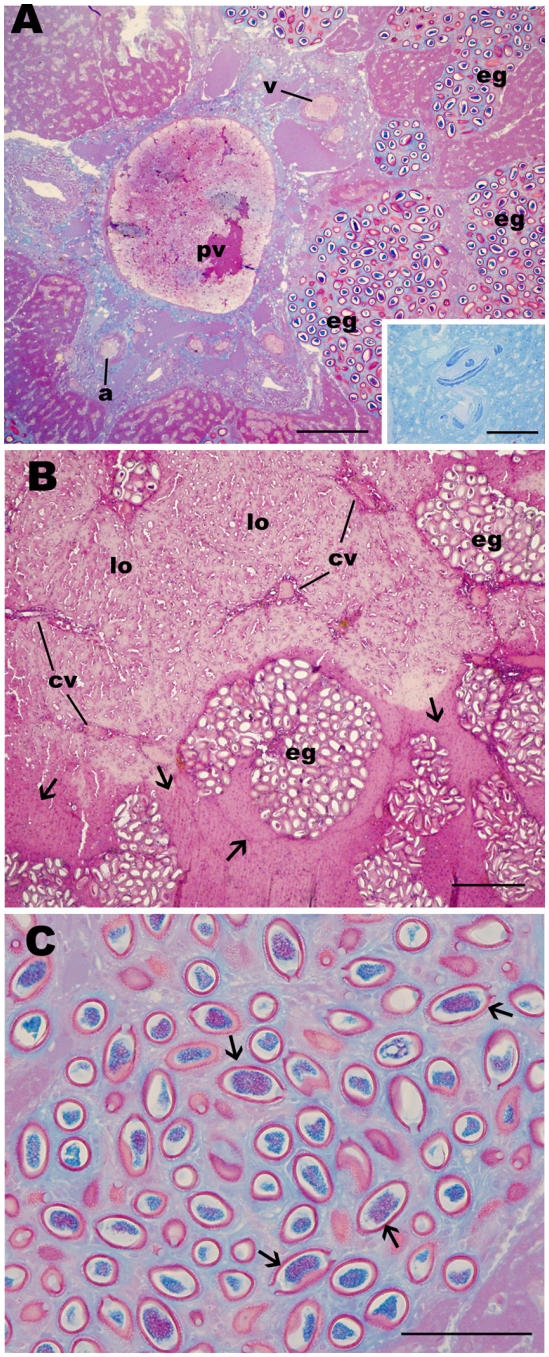

Figure 3. Histological features of the livers of *Rattus rattus* and *R. norvegicus* infected with *Calodium hepaticum*. A) Hepatic parenchyma characterized by the portal vein (PV), arterioles (a) and venules (v), and lobes compressed by the presence of adult parasites containing numerous eggs (eg) and larvae. Note the reaction of the cellular immune system of the host (Scale bar = 200 µm). *Insert*: Details of a larva infecting the hepatic parenchyma (Scale bar = 100 µm). B) Hepatic parenchyma with chronic infection characterized by the presence of fibrous tissue (arrows) and buildup of conjunctive tissue adjacent to the body of the helminth filled with eggs, with displacement of the centrolobular veins (cv) and deformation of the hepatic lobes. Note the presence of large numbers of eggs (eg) (Scale bar = 200 µm). C) Detail of the eggs (eg) with double-striated eggshells and bipolar opercula with plugs (Scale bar = 100 µm).

care professionals in Brazil. Thus, it has not been reported or investigated in humans or domestic animals.

Previous records from Brazilian cities have been restricted to Salvador, on the east coast, and São Paulo, in the south of the country, where human cases have also been recorded.

The findings of this study should serve to alert the local public health authorities in Belém (entrace municipalitie for the Amazon region) to isolated occurrences or possible outbreaks, reinforcing the necessity of investment in educational public health programs for the local population and health care professionals to create a vigilance barrier.

In broader terms, research into the occurrence of this helminth and its principal hosts will be necessary to map potential risks and identify the populations that are most vulnerable to this parasite.

The results of the present study reinforce the suggestion that the presence of synanthropic rodents and the zoonosis they transmit constitute an incentive for investments in a public sanitation system and the adoption of measures for the control of these agents and errant animals (stray dogs and cats), which can contribute to emerging and re-emerging diseases.

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