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Short communication

Prevalence of *Calodium hepaticum* (Syn. *Capillaria hepatica*) in house mice (*Mus musculus*) in the Azores archipelago

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ARTICLE INFO

Article history:

Received 23 June 2008

Received in revised form 23 October 2008

Accepted 3 November 2008

Keywords:

Calodium hepaticum

Mus musculus

House mice

Hepatic capillariasis

Rodents

Parasitic hepatitis

ABSTRACT

Calodium hepaticum (Syn. *Capillaria hepatica*) is a zoonotic liver nematode of mammals distributed worldwide. Rodents are believed to be the main reservoirs of this nematode. In this paper, prevalence of the parasite was analyzed in liver histological sections from 51 house mice (*Mus musculus*) caught in human-inhabited houses, from two localities (Furnas and Rabo de Peixe) on São Miguel island from the Azores archipelago (Portugal). Mean prevalence of infection was 19.6%, with 33.3% prevalence in Furnas and 4.1% in Rabo de Peixe ($P = 0.07$). No significant differences were found between the prevalence of infection and the age, body weight and the sex of mice. Hepatic lesions found were either acute and/or chronic stage and consisted of moderate to severe multifocal pyogranulomatous hepatitis with encapsulated eggs with typical bipolar plugs and moderate to severe necrotizing hepatitis consistent with larva tracks. Periportal inflammatory infiltration, hepatocyte regeneration and bile duct hyperplasia were also noted. In most cases, hepatic lesions occupied more than 50% of the liver, but despite severe lesions, in some mice, no signs of hepatic failure were noticed. The high rate of infection found in the present study suggests that house mice are an important reservoir for this parasite in the Azores and could have a role in human transmission.

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1. Introduction

Calodium hepaticum (Syn. *Capillaria hepatica*, Bancroft, 1893) is a nematode that parasitizes the liver of rodents and other mammals such as insectivores, rabbits, dogs, cats, cattle, and primates including humans (Hamir and Rupprecht, 2000; Spratt and Singleton, 2001; Lloyd et al., 2002; Ruas et al., 2003; Nakamura, 2005). Rodents are believed to be the main reservoirs of this nematode. Rat species of the genus *Rattus* are considered the main primary host and reservoir of this nematode and infection

to other mammalian occur incidentally due to ingestion of water or food contaminated with embryonated *C. hepaticum* eggs (Spratt and Singleton, 2001). The prevalence of *C. hepaticum* infection in the genus *Rattus* can be as high as 100% and has been reported worldwide (Farhang-Azad, 1977; Singleton et al., 1991; Ceruti et al., 2001). Only a few surveys have been reported in house mice (*Mus musculus*), perhaps surprisingly, given that this species cohabits closely with man and is very common in rural areas worldwide, including the Azores.

There have been around 37 reported cases of hepatic capillariasis in man, the majority of which were children from 1 to 5 years of age (Juncker-Voss et al., 2000). Infection in man can be subclinical but also fatal and described as a chronic fever syndrome, associated with eosinophilia, hepatomegaly, granulomatous hepatitis and liver fibrosis (Berger et al., 1990; Juncker-Voss et al., 2000).

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Unsanitary practices, poor hygiene, and the presence of dense rodent populations are all predisposing factors for human infections. Children or adults, with the habit of eating soil (geophagia and pica) as well as children with soil-to-hand-to-mouth contact, are especially at risk (Juncker-Voss et al., 2000). In man, this zoonosis is probably underestimated and mistaken for other granulomatous hepatitis or hepatic cancer mass (Klenzak et al., 2005). In areas infested with rats, especially within closed environments such as zoological parks, where the prevalence of the parasite in *Rattus norvegicus* can reach 100%, transmission to cohabiting mammals increases (Farhang-Azad, 1977; Landolfi et al., 2003; Redrobe and Patterson-Kane, 2005). Furthermore, such environments, with dense and infected rat populations, seem likely to predispose man to a higher risk of infection (Juncker-Voss et al., 2000).

The few surveys of *C. hepaticum* infection in house mice have shown infection rates from 5.4% to 24.6%, and there have also been negative in some areas (Farhang-Azad and Schlitter, 1978; Childs et al., 1988; Singleton et al., 1991; Spratt and Singleton, 2001). Although the prevalence of *C. hepaticum* in this species seems lower than in the *Rattus* genus, the role of house mice, a very abundant rodent in rural areas, in infection should be considered. The objective of this study was to assess the prevalence of *C. hepaticum* infection in wild house mice from two localities in S. Miguel Island of the Azores archipelago (Portugal) and to evaluate the associated pathological lesions.

2. Materials and methods

A total number of 51 house mice (*M. musculus*) were screened for *C. hepaticum* in liver histological sections. All mice were live-trapped in cages installed in human-inhabited houses, between spring and summer, in two different rural areas (Furnas and Rabo de Peixe), approximately 20 km apart from each other, in S. Miguel Island of the Azores archipelago. Furnas is a village located in the interior of an active volcanic crater and has a microclimate, characterized by extremely high humidity levels and rainfall, which cause soils to be very moist and higher in temperature due to volcanic degassing gases (Amaral et al., 2007). Rabo de Peixe is a village located in the north part of the island at a lower altitude where rainfall is less frequent and the ground more dry. Animals were euthanized in the laboratory, necropsied, and data regarding locality, sex, body and liver weight recorded. Mice were ranked by weight as sub-adult (≤ 12.5 g, $n = 11$ mice) and adult (> 12.5 –22 g, $n = 40$ mice) and sex (20 females, 30 males and 1 not determined). A total of 27 mice (10 females, 16 males and 1 not determined, 10 sub-adults and 17 adults) were caught in Furnas and 24 mice (10 females and 14 males, 2 sub-adults and 22 adults) in Rabo de Peixe. After detailed macroscopic examination, one to two liver sections with macroscopic lesions or one to two randomly selected sections in normal livers were included for histological examination. Collected liver samples were fixed in 10% buffered formalin and routinely processed for histological examination, embedded in paraffin, sectioned in 5 μ m and stained with haematoxylin and eosin (H&E). Diameter of the nematode was analyzed in transversal

sections. Infection status was statistically analyzed considering the variables locality, sex and categorical age group using 2×2 contingency tables by the χ^2 -test. The relation between infection status and body and liver weight was tested using one-way ANOVA and the Tukey–Kramer multiple comparisons test. Statistical analyses were performed with Stats Direct[®] and differences considered significant when $P < 0.05$.

3. Results

The diagnosis of *C. hepaticum* infection was made on the basis of the presence of eggs with typical bipolar plugs, which are characteristic of this parasite, or the presence of the adult nematode and larva in the liver parenchyma. A total of 10 mice were infected with *C. hepaticum* and showed mild to severe hepatitis. Mean prevalence of infection was 19.6% ($n = 10/51$) with prevalence of 33.3% ($n = 9/27$; 7 adults including 3 females, 3 males and 1 not determined and 2 sub-adults both males) in mice trapped in Furnas and of 4.1% ($n = 1/24$, male and adult) in Rabo de Peixe. There were statistically significant differences in the prevalence levels observed between the two locations ($P = 0.007$). Overall prevalence of infection was higher in adults ($n = 8/40$) than in sub-adults ($n = 2/11$) and, in males ($n = 6$) than in females ($n = 3$), but the differences were not statistically significant. Histopathological liver lesions consisted of acute necrotizing hepatitis associated with larval migration (initial development stage of the parasite) and chronic pyogranulomatous hepatitis in the presence of eggs (later stage of the liver infection). A total of seven mice had chronic lesions with loss of parenchyma lobular architecture due to moderate to severe multifocal to coalescing pyogranulomas, containing clusters of variable numbers of nematode eggs surrounded by neutrophils, eosinophils, lymphocytes and histiocytes, and scarce to moderate amounts of fibrous tissue (Fig. 1A). Rarely, granulomas had central necrosis, containing debris of eggs, with surrounding intense granulomatous inflammatory infiltration with epithelioid and multinucleated giant cells. In five mice the amount of the hepatic parenchyma affected by the pyogranulomas occupied more than 50% of the tissue section area. Additionally, there were moderate to intense periportal lymphoplasmacytic infiltration, dilatation of the portal tract hepatic vein, and hepatic parenchyma regeneration with moderate to intense bile duct hyperplasia, and hepatocytes karyomegaly and cytomegaly. Mild fibrosis and lymphoplasmacytic inflammatory infiltration surrounded adult nematodes embedded in the liver parenchyma. In three mice, adult nematodes were concomitant with egg clusters. In another three mice, the liver parenchyma had acute liver lesions with extensive track-like areas of coagulative necrosis associated with the nematode larva and with intense infiltration of inflammatory cells, mainly neutrophils. Periportal infiltration of lymphoplasmacytic cells, leukocytosis and hepatic regeneration and mild to moderate bile duct hyperplasia were also observed in most cases. No significant differences in liver weight and body weight were detected between infected and non-infected mice ($P > 0.05$). In addition, one mouse showed pyogranulomatous lesions that were not associated with

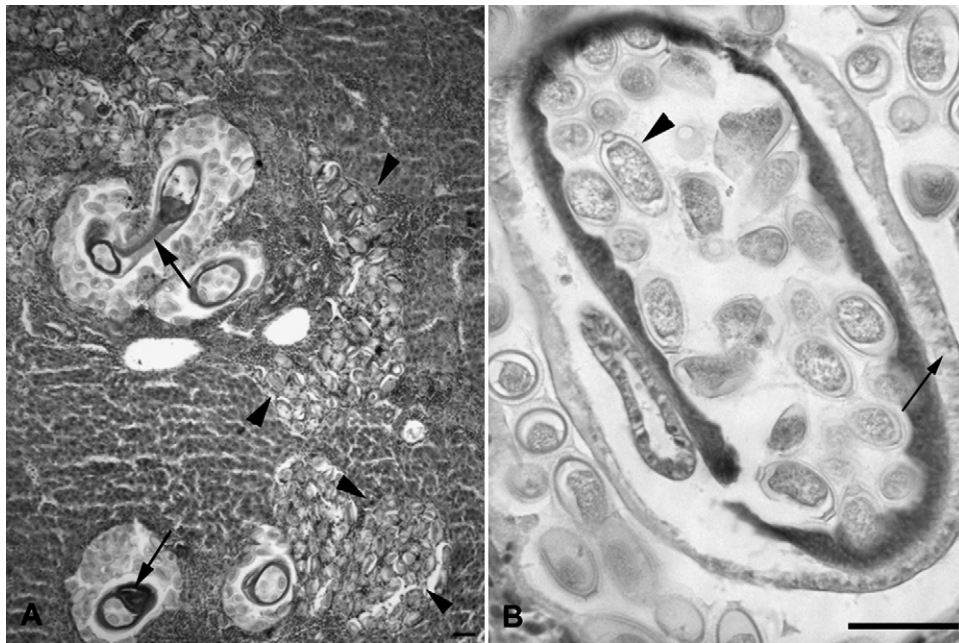


Fig. 1. Hepatic lesions and *Calodium hepaticum*. HE. (A) Multifocal hepatitis with an intense inflammatory cells infiltration surrounding clusters of eggs (arrowheads) and adult nematodes (arrows). Bar = 50 μm . (B) Adult nematode characterized by hypodermal bands, consisting of vacuolated cells with a nucleus (arrow) and with the genital tube full of barrel-shaped eggs with typical bipolar plugs and prominent radial striations in the outer layer of the shell (arrowhead). Bar = 50 μm .

eggs or with the presence of nematodes (this animal was not considered positive for the nematode infection). Eggs were barrel-shaped, unembryonated, had typical bipolar plugs and prominent radial striations in the outer layer of the shell, and measured on average 21 μm in width by 49 μm in length. Adult nematodes measured 88–154 μm in diameter, had a single genital tube, with a glandular wall and often containing eggs, an intestinal tract, a stichosome oesophagus and several hypodermal bands, consisting of vacuolated cells with a nucleus, characteristic of aphasmid nematodes (Fig. 1B).

4. Discussion

To our knowledge this is the first report of infection for *C. hepaticum* in Portugal. Our results show that a high prevalence of *C. hepaticum* infection can be found in house mice, especially in one of the areas analyzed. Moreover, we also found that infected house mice can have severe hepatic lesions without evidence of hepatic failure and/or death. Taken together our results indicate that the house mouse is a potential reservoir for this nematode infection and therefore, a health risk for hepatic capillariasis in man.

The main reservoirs of *C. hepaticum* are thought to be *Rattus rattus* and *R. norvegicus*, since high prevalence have been registered in these species (Ceruti et al., 2001; Spratt and Singleton, 2001). In comparison to rats, the few existing surveys of prevalence in house mice suggest that this species is less likely to be infected with *C. hepaticum* (Farhang-Azad and Schlitter, 1978; Childs et al., 1988; Singleton et al., 1991; Spratt and Singleton, 2001). However, under natural conditions we found that the

range of infection of house mice by *C. hepaticum*, although variable, could reach high rates, similar to those detected in the *Rattus* species. Thus, the house mouse should also be considered a source of this parasite infection for other animals and potentially for humans.

The life cycle of *C. hepaticum* is direct, with no intermediate host required, and needs the host's death for transmission (Spratt and Singleton, 2001). Eggs are released from hepatic tissue via cannibalism, predation by vertebrates, or decomposition of the host and disseminated throughout faeces of predators, such as dogs and cats, in the environment. To become infectious, eggs have to become embryonated, requiring a 5–7 week time period in the soil, under particular temperature and humidity conditions. After ingestion of embryonated eggs, the hatched larva penetrates the intestinal wall and migrates via the mesenteric and portal veins to the liver, where molts develop into the adult nematode, within approximately 20 days post-infection. Female adult worms lay clusters of eggs in the hepatic parenchyma and die within 60 days after infection (Spratt and Singleton, 2001). Requirements described for the full transmission of this nematode to other mammals are a high density of rodents, moist soil and poor hygiene (Spratt and Singleton, 2001). A possible explanation for the higher prevalence of the nematode in Furnas compared to Rabo de Peixe could be the particular microclimate of moist soils and high humidity that better enables the transmission of the parasite.

The pathogenicity of this parasite in rodent species is considered low, however experimental inoculation of the parasite, both in rat and mice, have demonstrated its potential to cause hepatic failure and death of the host

(Vollerthun et al., 1974; Spratt and Singleton, 1986). *C. hepaticum* is able to reduce the host survival by 5–10% (Singleton and Chambers, 1996), but rats may be heavily infected without obvious clinical signs, which may indicate partial resistance by the host (Farhang-Azad, 1977). Hepatic failure and the host death are associated with increased levels of liver enzymes, eosinophilia, hepatocyte death, severe granulomatous hepatitis and cirrhosis (Luttermoser, 1938; Solomon and Soulsby, 1973; Spratt and Singleton, 1986). Some differences in the lesions caused by this parasite have been observed in rats and mice (De Andrade and Andrade, 2004). The granulomatous hepatic lesions found in the present study were consistent with those observed in previous reports (Borucinska et al., 1997; Hamir and Rupprecht, 2000; Ceruti et al., 2001; Landolfi et al., 2003; De Souza et al., 2006). However, acute necrotizing lesions associated with larva migration were not constantly described for this infection, nor has bile duct hyperplasia. Lesions found in our study were either acute and/or chronic, which were associated with the developmental stage of the nematode. Despite the severity of lesions found we did not detect signs of hepatic failure in the mice in our study. However, it is possible that the more severe hepatitis lesions could have resulted in hepatic dysfunction.

In conclusion, our results indicate that house mice are a potential reservoir for *C. hepaticum* and are a potential health risk for human capillariasis in the Azores.

Acknowledgements

This study was supported by CIRN (Centro de Investigação em Recursos Naturais, Universidade dos Açores), and DRCT (Direcção Regional da Ciência e Tecnologia, Governo dos Açores). Ana Resendes was supported by a Postdoctoral grant from FCT (FRCT/2008/112/004). We are grateful to Elena Obón for critical review of the manuscript and for Paulo Melo for excellent technical assistance.

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