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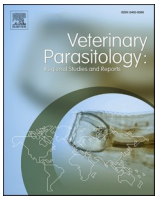

Regional report

Zoonotic nematode in the city of La Plata, Argentina: Report of a case of *Calodium hepaticum* in *Rattus rattus*

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ABSTRACT

This study describes a case of *Calodium hepaticum* (Trichinellida: Capillariidae) infection in an adult rat (*Rattus rattus*) from the periurban area of the city of La Plata in the province of Buenos Aires, Argentina. The rat was found with neurological signs (ataxia, lethargy, and episodes of unresponsiveness) in the food storage of a goat production facility. The liver was observed with hepatomegaly and diffuse and irregular yellowish-white spots appearing in striae or small nodules on the external surface and inside the liver. Subsequent microscopic and histopathological studies were performed. Eggs were observed by direct microscopy of the impression smear of liver tissue. A multifocal granulomatous tissue reaction with different stages of fibrocellular tissue was observed in the liver parenchyma. The granulomas contained adults and degenerated eggs delimited by an intense infiltrate of mononuclear cells. Macro and microscopic observations and histopathological liver lesions were compatible with *C. hepaticum* infection. To our knowledge, this is the first confirmation of *C. hepaticum* infection in *R. rattus* in Argentina, increasing the host record of this parasite and a new record of distribution in goat production systems in the country.

1. Introduction

Rodents of the genus *Rattus* (*Rattus rattus* Linnaeus 1758 and *Rattus norvegicus*; Berkenhout 1769) have a worldwide distribution, and their main habitat is urban and rural areas in relation to humans, with an enormous capacity for adaptation (Simões et al., 2014). Because of this, it is common to find them in livestock production systems and farms from different species where structural and environmental conditions are favourable (Gómez Villafañe et al., 2008; Lovera et al., 2015; Lovera et al., 2019). Rodents play an active role in the transmission of a large number of zoonoses, such as toxoplasmosis, leptospirosis and hymenolepiasis, among others, considering them a public health risk (Fuehrer et al., 2011; Fitte et al., 2021; Brar et al., 2021).

Calodium hepaticum Moravec 1982 (Trichinellida: Capillariidae) (syn. *Capillaria hepatica*) is a zoonotic nematode that parasitises the hepatic parenchyma of rodents and other mammals. Successful transmission of the parasite requires the death of the host, decomposition and allowing eggs to embryonate and become infective. Additionally, the host tissues could be consumed by predator/canibal/scavenger animals which excrete eggs in their feces (intestinal passage) contaminating the environment (Fuehrer et al., 2011; Simões et al., 2014; Fantozzi et al., 2022). Animals and humans can become infected by ingesting embryonated eggs in water, soil or contaminated fruits and vegetables (Fuehrer et al., 2011; Moreira et al., 2013; Fantozzi et al., 2018, 2019). In Argentina, the presence of *C. hepaticum* eggs was recorded in faecal material of *R. norvegicus* (brown rats) in the city of Buenos Aires (Hancke et al., 2011) and a high prevalence of *C. hepaticum* has been found in Argentina in sigmodontine rodents which often cohabit with *R. norvegicus* and *R. rattus* (Fantozzi et al., 2018, 2022). In *R. rattus* from South America, *C. hepaticum* has only been reported in urban areas of

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Brazil, however, this parasite is found frequently and at high prevalence (>40%) in rats (*Rattus* spp.) worldwide, and most of the animals showed no clinical signs (Simões et al., 2014; Fantozzi et al., 2018; Brar et al., 2021). In Argentina, there is currently no information on reports of *C. hepaticum* in *R. rattus* and its association with goat production facilities.

The aim of this work was to report the finding of *C. hepaticum* in *R. rattus* (roof rat) in the vicinity of a goat farm located in the periurban area of the city of La Plata in the province of Buenos Aires, Argentina extending the known distribution and host record.

2. Case presentation

A specimen of *R. rattus* was found inside the food storage room of a goat farm, located in the periurban area of the city of La Plata, province of Buenos Aires, Argentina (−34.91976, −57.95386), in the month of February 2023. The rat showed neurological sign, such as ataxia, lethargy and severe episodes of unresponsiveness short before death. The production system of the farm is essentially dual-purpose (milk and meat production). The farm has a storehouse where the feed (fodder grain and balanced feed) is stored for supplementation of the goats. *Toxoplasma gondii* seropositive goats have been recorded on the same farm and cats are present (Steffen et al., 2021). The specimen was capture and identified by measuring full body and tail length (March, 1994), the necropsy was carried out in the Laboratory of Immunoparasitology (LAINPA) under the corresponding biosecurity measures, and in accordance with the Ethics Committee of the University. Initially, a macroscopic examination of the organs was performed. No lesions were observed in the central nervous system or other organs, with the exception of the liver. Due to the neurological signs of the rat, serological and parasitological diagnosis of *Toxoplasma gondii* and *Neospora caninum* were performed (Dubey, 2010). No specific anti-*T. gondii* and anti-*N. caninum* antibodies were found in the serum sample. No tissue cysts were observed in the homogenized central nervous system (CNS) tissue, and neither were tachyzoites observed in the impression smear of lung tissue by direct microscopy. Faecal material was also collected and examined for helminth eggs and protozoan eggs cyst/oocysts, with negative results in both cases.

Liver hepatomegaly was observed with diffuse and irregular yellowish-white patches appearing in striae or small nodules on the external surface and inside the liver. The lesions observed showed massive extension, affecting more than half of the liver, compatible with that described by Ceruti et al. (2001) for severe or grade 3 (Fig. 1). Parasitic forms, adults and eggs were observed inside the lesions (Fig. 2a and b). Adults and/or parts of adults recovered from the lesions were clarified in lactophenol, studied under the light microscope, and identified according to Wright (1961). A portion of liver tissue was fixed in 10% buffered formalin and sent to the Centre for Parasitological and Vector Studies (CEPAVE) for histopathological diagnosis. *C. hepaticum* infection was confirmed by the presence of adults and oval eggs with bipolar prominences or caps and radial striations on the membrane (Fig. 2b and 3d). The range of egg measurements was 56.3–40.4 μm long x 28.2–21.8 μm wide (with a mean of 51.1 μm × 24.7 μm). The eggs showed the typical morphological characteristics of this species, as described by Ceruti et al. (2001). The morphological characteristics observed in the adult females (Fig. 2a) agree with the description of Wright (1961).

Fig. 1. Macroscopic view of the visceral side of the left lateral lobe of the *R. rattus* liver with nodular lesions (arrow) showing multifocal, sinuous irregular yellowish-white spots under Glisson's capsule.

All liver lesions harboured *C. hepaticum* eggs inside (Fig. 3a and d) and some lysed dead parasites (Fig. 3b). In addition, the uteri of adult females contained intact or degenerated eggs was observed (Fig. 2a and 3d). In the analysis of histopathological sections of the infected liver, a multifocal granulomatous tissue reaction with different stages of fibrocellular remodelling tissue was observed (Fig. 3a and b). The granulomas contained intact and degenerated *C. hepaticum* eggs delimited by an intense infiltrate of mononuclear cells (monocytes and macrophages) (Fig. 3c and d). In the liver parenchyma, marked parenchymal remodelling with septal fibrosis, necrotic and fibrotic tissue, and the accumulation of connective tissue adjacent to the body of the degenerated dead parasites were observed (Fig. 3b). The histopathological findings in the liver were in agreement with observations from other studies in naturally infected rats (Santos et al., 2001; Duque et al., 2012; Moreira et al., 2013; Simões et al., 2014; Brar et al., 2021). Following this study, another rat of the same species (*R. rattus*) from the same goat farm was necropsied and *C. hepaticum* was found.

3. Discussion and conclusions

Globally, *C. hepaticum* is often highly prevalent in rat species (*Rattus* spp.) in urban and rural environments, despite this, studies involving urban rodents are scarce compared to their importance to public health (Brar et al., 2021; Fitte et al., 2021). There are numerous reports of *R. norvegicus* in South American countries such as Chile, Colombia, Brazil, and Argentina, therefore, it seems to be the main host species of *C. hepaticum*. Only *C. hepaticum* has been reported on *R. rattus* in South America, in several Brazilian districts (Moreira et al., 2013; Simões et al., 2014; Fantozzi et al., 2018; Fitte et al., 2021). Additionally, in Argentina, there are also reports describing the presence of other parasites and zoonotic pathogens in urban rodents (Fitte et al., 2021). Even, studies have been conducted on the presence of different rodent species in poultry, pig and dairy farms, and their distribution according habitat characteristics and management strategies in these agricultural production systems were identified (Gómez Villafañe et al., 2008; Lovera et al., 2019). Our case study reported the first record of clinical infection with *C. hepaticum* in *R. rattus* in Buenos Aires, Argentina and in a goat farm. The characteristics associated with this farm coincide with the habitat preferences of *R. rattus* described by Lovera et al. (2019), among intensive production systems in central Argentina. As previously mentioned, the farm has availability of food and shelter which are the main factors for the settlement of rodents (Lovera et al., 2019). In livestock production systems the presence of rodents is associated with damage to animal feed by consumption and contamination, structural damage to buildings and equipment, and transmission of diseases, thereby resulting in reduced production (Gómez Villafañe et al., 2008; Lovera et al., 2015). In urban areas, the transmission of *C. hepaticum* to humans has been considered to be related to the presence of rodents (*Rattus* spp.), due to high prevalence of natural infection, and cannibalistic habits that would explain the high transmission of the parasite among rodents and environmental contamination with eggs (Moreira et al., 2013; Fantozzi et al., 2022). Further studies have suggested that domestic animals (cats and dogs) may also contaminate peridomestic

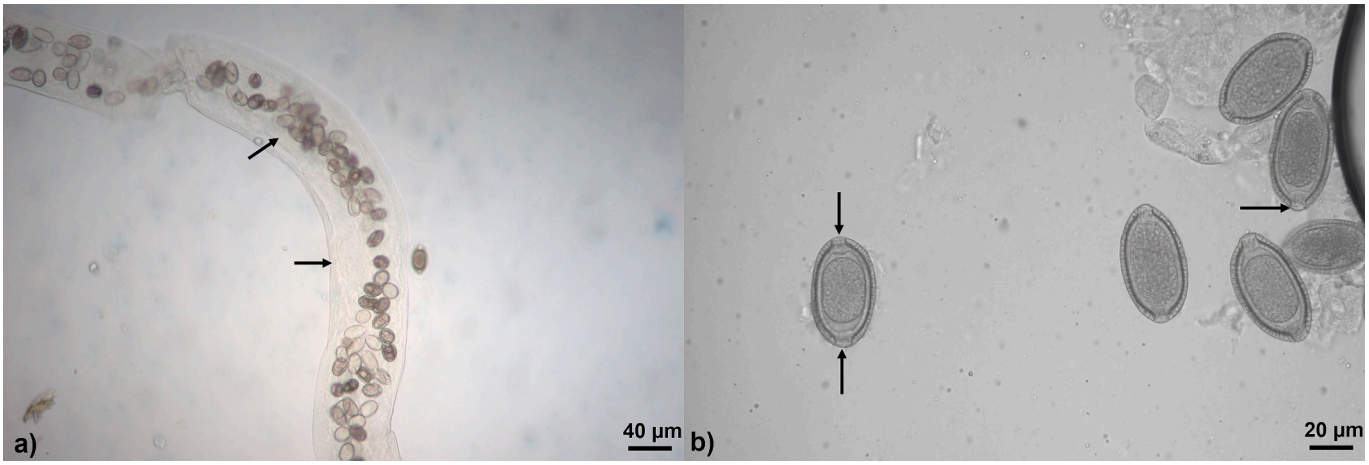

Fig. 2. a) Lateral view of a portion of a *C. hepaticum* female specimen with uterus filled with eggs (arrows), cleared in lactophenol (10×). b) Oval/elliptical eggs with a double striated shell and bipolar caps (arrows) (40×).

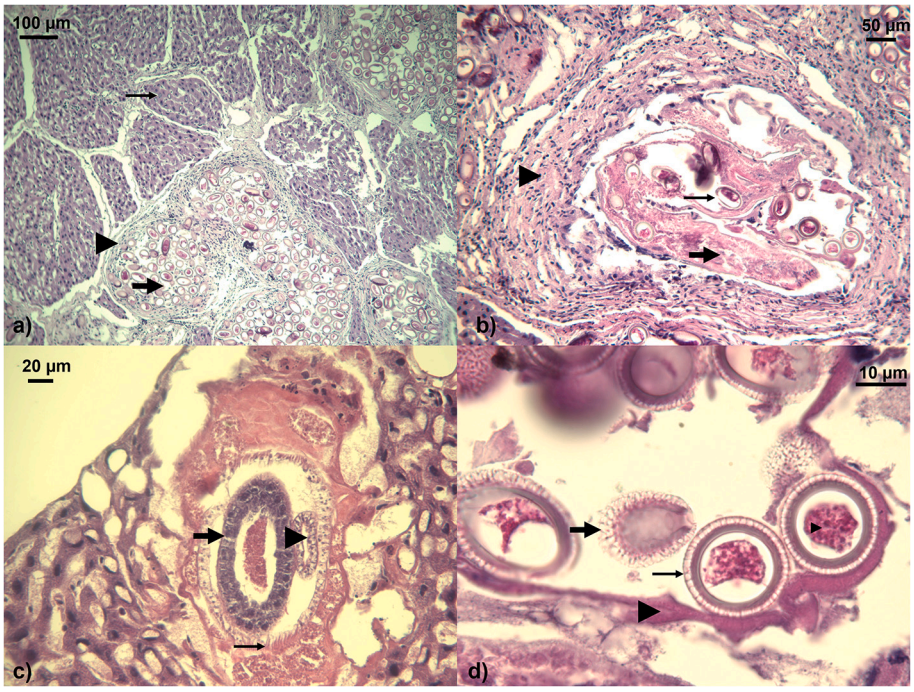

Fig. 3. Histological features of *R. rattus* liver infected by *C. hepaticum*. a) Liver parenchyma with compressed/deformed lobules (slim arrow) by foci of fibrous (arrowhead) and inflammatory tissue surrounding numerous eggs (thick arrow) (10×). b) Reaction of fibrous and inflammatory tissue (arrowhead) and accumulation of connective tissue adjacent to the lysed body of a dead helminth (thick arrow) with eggs inside (slim arrow) (20×). c) Cross section of *C. hepaticum* in liver tissue, showing sections of the intestine (arrowhead), the uterus (thick arrows), and the muscular layer (slim arrow) (40×). d) Oval/elliptical eggs of *C. hepaticum* with thick wall (bilayer) (slim arrow) formed by proteins that form radial striations in the membrane (thick arrow), with granular eosinophilic content (small arrowhead). Note the uterus wall containing the eggs (large arrowhead) (100×).

soil with infected feces after eating small rodents, or infected liver from other mammals (Gonçalves et al., 2012), increasing the likelihood of incidence of this parasitosis in humans (Fuehrer et al., 2011). Human hepatic capillariasis presents with mild to severe clinical signs and symptoms, with possible fatal outcome (Wang et al., 2013).

More than 180 mammalian species (including humans) are hosts of *C. hepaticum* (Fuehrer et al., 2011), causing severe liver disease, with persistent fever, liver damage, impaired liver function, liver fibrosis, hepatomegaly, eosinophilia and even death (Zhou et al., 2020). In rodents, after ingestion, embryonated eggs hatch in the intestine and release first stage larvae that penetrate the caecal wall and reach the liver via portal veins. Larval migration within the liver parenchyma results in areas of liver necrosis (Ceruti et al., 2001; Moreira et al., 2013; Fantozzi et al., 2018; Brar et al., 2021).

The macroscopic features such as lesions in the liver of *R. rattus* presented multifocal yellowish-white striae and spots are consistent with those described for *C. hepaticum* for *Rattus* spp. (Moreira et al., 2013; Simões et al., 2014; Fantozzi et al., 2018; Brar et al., 2021). The histopathological lesions identified, presented foci of granulomatous hepatitis together with hepatocellular necrosis, vascular congestion, and cellular infiltrate consistent with described cases of hepatic capillariasis (Ceruti et al., 2001; Brar et al., 2021; Fantozzi et al., 2022). Several authors claim; that rats are the main hosts, but despite this, signs of disease are hardly seen, even when they are heavily infected (Fantozzi

et al., 2019). Although no molecular analysis of the parasite was performed in these studies, PCR (18S rRNA gene) is now also available for the diagnosis of *C. hepaticum* (Fantozzi et al., 2018; Zhou et al., 2020). In our report, neurological clinical signs (ataxia, lethargy, and episodes of unresponsiveness) of severe intensity were evident before specimen capture. Although no toxicological studies have been carried out, it is concluded that the specimen under investigation has not been poisoned, whereas the most widely used rodenticides in the country are composed of long-acting anticoagulants, and the specimen did not show clinical signs compatible with haemorrhagic manifestations, such as haematuria, petechiae, ecchymosis, melena, intracranial haemorrhage (Popov Aleksandrov et al., 2018). The neurological clinical signs present in the rat could probably be due to hepatic encephalopathy caused by the massive liver lesions and impaired hepatocellular function involved in the pathogenesis of this disease. Although liver function tests, such as biochemical profile, blood gas analysis and abdominal imaging are necessary to confirm hepatic encephalopathy, they could not be performed in this study (Gow, 2017).

In a previous study carried out in the locality of La Plata, in the province of Buenos Aires, Argentina >100 rodents were studied without finding the presence of this parasite (Fitte et al., 2021), in this context, this is the first report of *C. hepaticum* infection in *R. rattus* in Buenos Aires, Argentina, and a new distribution record in a goat production system. This finding is consistent with reports of different rodent species in different production systems, such as farms, dairy farms and orchards, given the favourable conditions for their settlement. Furthermore, the confirmed findings of *C. hepaticum* in both specimens may suggest that the life cycle and transmission of the parasite is present and maintained in the local rat population. Therefore, the presence of infected rodents near production systems poses a substantial risk to animal and human health. Due to the scarce reports of parasitosis in rodents and its association with these systems, it is necessary to continue investigating the incidence of this zoonotic nematode and its different hosts. Finally, it is necessary to adopt an integrated control with proposals and strategies based on the understanding of the biology and ecology of the species involved, where different types of measures are integrated under the concept of One Health to mitigate this problem.

Founding

Financial support was obtained by a young researchers grant awarded by UNLP – 2022 (Kevin Denis Steffen).

Author contributions

Kevin Denis Steffen conducted the necropsy, microscopy and wrote the manuscript. Carina Basset was involved in the microscopy and image obtention. Ruben Arias collected the specimen and is supervising the farm management. Gastón Moré identify the species and wrote the manuscript. Maria del Rosario Robles performed the histopathological studies and parasite identification. Juan Unzaga supervised the study. All authors revised the final version of the manuscript.

Ethical statement

We are pleased to submit a manuscript entitled: "Zoonotic nematode in the city of La Plata, Argentina: Report of a case of *Calodium hepaticum* in *Rattus rattus*" by Steffen, KD; Basset, C; Arias, RO; Moré, G; Robles, MdR; Unzaga, JM, to be considered for publication in *Veterinary Parasitology: Regional studies and reports*. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. The material is original and is not under consideration for publication elsewhere.

The animal procedures performed were approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL), under the Secretary of Science and Technology of the Faculty of Veterinary Sciences of the National University of La Plata.

Furthermore, we confirmed that no generative artificial intelligence (AI) and AI-assisted technologies were used in the writing process.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

Complete specimen of *R. rattus* was deposited in the Mastozoological Collections of the *Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"* (MACN).

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