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Short Communication

Occurrence of *Capillaria* sp. in the liver of sheep (*Ovis aries*) in a slaughterhouse in the state of Acre, Brazil

Ocorrência de *Capillaria* sp. em fígado de ovino (*Ovis aries*) em um abatedouro no estado do Acre, Brasil

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Abstract

Although sheep farming has grown in the state of Acre over the past four decades, little is known about occurrences of helminthiases in the herds of this region. The objective of the study was to assess the occurrences of non-intestinal helminthiasis among sheep slaughtered in Rio Branco. A total of 110 sheep livers were inspected from two slaughter batches (july 2014 and march 2015) in a slaughterhouse in Rio Branco. Livers with macroscopic lesions were photographed and were then subjected to histopathological analysis under an optical microscope. The macroscopic lesions showed small nodes with inflammatory characteristics and areas of fibrosis, which appeared to be calcified, thus suggesting a granulomatous reaction. Of the 110 evaluated livers, we noticed 110 nodules in total; these nodules have an average size of 0.5 cm. The histopathological analysis showed alterations to the architecture of the hepatic lobe, with multiple foci of necrosis and polymorphonuclear cells. Two samples revealed the presence of helminths from Nematode class and *Capillaria* sp. eggs identified by the typical morphology and morphometry. This seems to be the first report of *Capillaria* sp. in sheep livers in Brazil, and it serves as an important alert regarding animal health surveillance and control and regarding the *Capillaria* sp. zoonotic role in humans.

Keywords: Histopathology, hepatic lesions, helminths, nematodes, bright-field microscope, Amazon rainforest.

Resumo

Embora a ovinocultura tenha despertado o interesse de criadouros no estado do Acre nas últimas quatro décadas, pouco se conhece sobre a ocorrência de helmintoses no plantel de ovinos dessa região. O objetivo do presente estudo foi avaliar a possibilidade de ocorrência de helmintíases não intestinais entre ovinos abatidos no município de Rio Branco. Foram inspecionados 110 fígados de ovinos em dois abates (julho de 2014 e março de 2015) em um abatedouro no município de Rio Branco. Fígados com lesões macroscópicas foram fotografados com posterior análise histopatológica por microscopia de luz. Nas lesões macroscópicas foram encontrados pequenos nódulos apresentando características inflamatórias com áreas de fibrose, aparentemente calcificadas, sugerindo uma reação granulomatosa. Dos 110 fígados avaliados, observou-se 110 nódulos no total; estes nódulos têm um tamanho médio de 0,5 cm. A análise histopatológica mostrou alterações na arquitetura do lóbulo hepático, com múltiplos focos de necrose, além da formação de abscessos hepáticos constituídos por polimorfonucleares. Duas amostras revelaram a presença de helmintos da Classe Nematoda e ovos de *Capillaria* sp. identificados pela morfologia típica e morfometria. Esse resultado parece ser o primeiro registro de *Capillaria* sp. em fígado de ovino no Brasil, o que é um importante alerta para a vigilância no controle sanitário animal e o seu papel zoonótico para humanos.

Palavras-chave: Histopatologia, lesões hepáticas, helmintos, nematóides, microscopia de campo claro, floresta Amazônica.

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Introduction

Sheep farming has been shown to be an attractive investment in developing countries (LEROY et al., 2015). Brazil has followed this trend with herds distributed mainly in the northeastern and southern portions of the country (RIBEIRO & GONZÁLEZ-GARCÍA, 2016). Over the past four decades (1985-2015) there has been high growth in the northern region of Brazil (IBGE, 2015), particularly the use of the Santa Inês breed because of its high productivity and hardiness (RIBEIRO & GONZÁLEZ-GARCÍA, 2016).

Helminthiases are the main causes of loss of production in sheep herds (MACIEL, 2014). The nematode *Haemonchus contortus* is the most epidemiologically important gastrointestinal helminth among sheep in tropical and subtropical regions in the world (MAVROT et al., 2015). Data from Brazil confirm the importance of *H. contortus* (CARDOSO et al., 2012; ATAÍDE & CANSI, 2013; FERREIRA et al., 2015; VIEIRA et al., 2014; WILMSEN et al., 2014), particularly for the Santa Inês breed of sheep in the state of Rio Grande do Norte (SOUZA et al., 2012). On the other hand, ruminants may also be infected by other gastrointestinal nematodes that are considered to be less epidemiologically important, such as *Capillaria bovis* Schnyder, 1906. This species has been reported in cervids in France (JUSTINE & FERTÉ, 1988, 1989), Canada (DIES & COUPLAND, 2001) and Turkey (BOLUKBAS et al., 2012), wild deer in Norway (DAVIDSON et al., 2014) and sheep in India (JAIN & KAMLAPUR, 1969).

In Brazil, infection by *C. bovis* (syn. *Aonchotheca bovis*) (FREITAS & MENDONÇA, 1961) was reported in cattle in different states, and specifically in the small intestine of *Ovis aries* in the microregion of Jaboticabal, in the northwestern region of the state of São Paulo (MACIEL, 2014). Although capillarids have a wide range of mammal hosts (rodents, lagomorphs, ruminants, birds, canids, fishes, non-human primates and humans), data from the literature show that these are predominantly parasites of rodents (FUEHRER, 2014a). Moreover, zoonotic potential has been reported as particularly relevant for *C. hepatica* (FUEHRER, 2014b) and *C. bovis* in ruminants in Poland (GAŁĘCKI et al., 2015).

Definitive hosts become infected by ingesting embryonated eggs spread into the environment. First stage larvae (L1) hatched in the small intestine, migrate via the portal system to the liver, where adults worms mate. Gravid females lay hundreds of unembryonated eggs in the surrounding liver parenchyma, which are unable to leave the host (true infection). The life cycle progression requires the death of the host, in which decomposed liver releases unembryonated eggs into the environment. The alternative transmission pathway includes predators, scavengers and cannibals animals, which also release unembryonated eggs. Because such nematodes are soil-transmitted helminths, unembryonated eggs require environmental factors (soil texture, pH and temperature) to progress to the embryonic stage (GONÇALVES et al., 2012).

Over the last decades, a novel mode of transmission has been reported, when humans feed meat of wild mammals in Brazil (CAMARGO et al., 2010; KLISIOWICZ et al., 2014; QUADROS et al., 2016). Eggs pass away the gut and are released in stool similar to predators (spurious infection) (COIMBRA & MELLO, 1981; SOARES et al., 2011; FUEHRER, 2014a).

Liver capillariasis is characterized by small milky spots, focally distributed in the surface or parenchyma. The histopathological features include hepatic granuloma and eventually fibrosis (ANDRADE & ANDRADE, 2004; JEONG et al., 2008; GABAN et al., 2010). However, little is known about the histopathological situation of natural capillarid infections among ruminants (NAKAMURA, 2005). These last authors used the term parasitic bovine hepatitis for multiple small lesions with fragments of worms similar to capillarids. Their report was similar to what was described for rodents naturally infected by *C. hepatica* (JEONG et al., 2008; SINGLA et al., 2013; MOREIRA et al., 2013). Similar to other dwelling-liver helminth (*Fasciola hepatica* and metacestodes of tapeworms), the inspection of carcasses of meat could detect cases of capillariasis (UNGAR et al., 1990; TESSELE et al., 2013).

The objective of the present study was to assess the possibility of occurrences of non-intestinal helminthiasis among sheep slaughtered in the municipality of Rio Branco, the capital of the state of Acre.

Materials and Methods

Sheep livers (n=162) were inspected for the presence of macroscopic lesions at a slaughterhouse in the municipality of Rio Branco (9° 52' 47" S; 67° 47' 10" W), during two slaughter batches (July 2014 and March 2015). This slaughterhouse, which is located in the state capital, receives animals from the municipalities of Rio Branco, Bujari, Sena Madureira, Feijó, Senador Guiomard, Capixaba, Acrelândia, Plácido de Castro, Porto Acre, Xapuri, Brasiléia and Epitaciolândia (Figure 1).

The animals were slaughtered in accordance with the regulations governing humane slaughter. The macroscopic evaluation of the livers took place after a sanitary evaluation. Livers that presented alterations were photographed using a digital camera (Nikon, model DS-Fi1, China), fixed in 10% neutral formalin and sectioned into pieces. Routine histopathological processing of the samples and histopathological analyses were conducted at the Laboratory for Helminth Parasites of Vertebrates of the Oswaldo Cruz Institute (FIOCRUZ, Rio de Janeiro) and at the Romero Lascasas Porto Helminthology Laboratory of the State University of Rio de Janeiro (UERJ).

Slides were stained with hematoxylin and eosin (H&E) and picrosirius. The histopathological analysis on the material was carried out using a bright-field microscope (Nikon, model Eclipse E200, China). The images were processed in a digital image analyzer equipped with the Nis Elements AR software (Nikon, USA).

Capillaria sp. eggs that were found in the hepatic parenchyma were subjected to morphometric analysis (total length and width), considering the shape of the eggs and the bulge of polar plugs. The morphometric analysis was performed through an ocular micrometer (Nikon, China). All the measures found were compared with those already existing in the scientific literature (FUEHRER, 2014b).

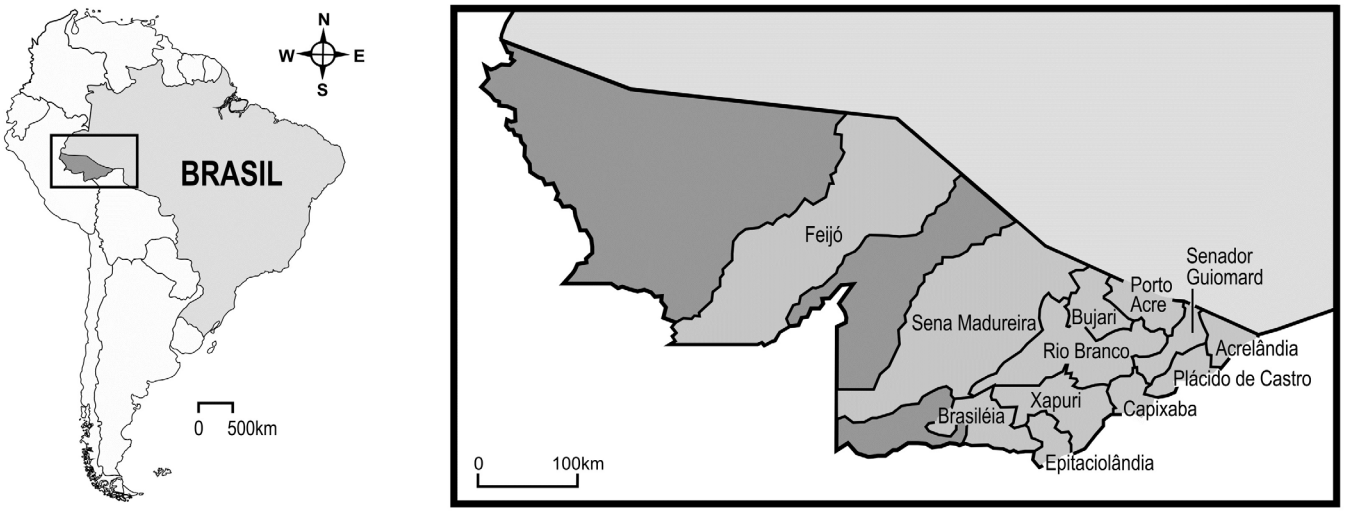

Figure 1. Municipalities from where the sheep at the slaughterhouse in the city of Rio Branco, Acre, Brazil, originated. Source: Illustration produced by Heloisa Diniz, Image Treatment and Production Service, IOC/Fiocruz-RJ, Brazil.

Results

Among the 162 animals slaughtered, the livers of 110 animals (67.9%) presented macroscopic lesions, of which 63 (57.2%) were in males and 47 (42.7%) were in females. The macroscopic lesions in the macroscopic evaluation, well delimited masses were observed presenting elevations to the external surface of the liver, irregular, little encapsulated, firm and subcapsular, about 2-5cm in diameter with a grayish-white coloration, therefore, lighter than the liver around them (Figure 2A).

The histopathological findings of the presence of *Capillaria* sp. showed a variety of results from the disintegration of the parasite and the presence of its eggs in the liver. In regions close to the eggs of the parasite, necrotic-inflammatory focal lesions with lympho-histoplasmacytic and multifocal eosinophilic infiltrate were observed (Figures 2B-D).

Other histopathological findings, dispersed in the hepatic parenchyma and linked to the presence of *Capillaria* sp., were verified: presence of pseudolobules formed by groups of hepatocytes with strings arrangement without defined lobular architecture, surrounded by fibrous tissue originating from the portal spaces, containing numerous proliferative bile ducts, multiple necrosis foci, in addition to the formation of hepatic abscesses, consisting of polymorphonuclear cells, with poorly delimited and irregular margins (Figure 2B).

We observed a necrotic central lesion, distributed in several areas in the hepatic parenchyma, suggesting debris of *Capillaria* sp. and / or remains of dead eggs, characterizing a granulomatous reaction. Dystrophic calcification can be observed in some necrotic central lesions and the presence of palisade cells around the granulomatous reactions, especially in those that did not yet present fibrous encapsulation (Figure 2C).

The histological sections revealed the presence of cylindrical helminths, with a pseudocoelom, a muscle layer in the body wall with longitudinal fibers, a covering cuticle and a triradiate pharynx, thus indicating a representative of the phylum Nematoda (Figures 2D, E).

The hepatic parenchyma of one sample presented a cluster of eggs of the genus *Capillaria* sp., which presented an oval shape, striated capsule and non-profuse bipolar operculum (Figure 2F). The morphometry of the eggs (n = 10) indicated variations in length between 47.5 and 50 μm (mean: 48.5 ± 1.29 μm) and in width between 22.5 and 25 μm (mean: 24.5 ± 1.05 μm).

Other histopathological findings, bridged portal fibrosis, thick septa of connective tissue, frequently connecting a portal space to another, formation of long thin fibrous septa overlying the central vein between them, accumulation of fat in the form of rounded globules of varying sizes in the hepatocyte cytoplasm, classified as macro and microsteatosis. In addition, chronic inflammatory mononuclear tissue composed of macrophages, lymphocytes and fibroblasts was found, delimited by a fibrous capsule, characterizing a granulomatous reaction.

Discussion

The objective of the present study was to assess the possibility of occurrences of non-intestinal helminthiasis among sheep slaughtered in the municipality of Rio Branco, the capital of the state of Acre. Over the past four decades there has been a significant increase in sheep farming in this region and the animals slaughtered in Rio Branco originated from several municipalities in this state.

Macroscopic hepatic lesions characterized as small inflammatory nodules with areas of fibrosis, which appeared to be calcified, were suggestive of the presence of granulomas. Although hydatidosis and fasciolosis are included in this situation (ROBERTS, 1982; VECHIATO et al., 2011), there were no indications of infection by these parasitic agents in the present study. On the other hand, macroscopic hepatic lesions are a common finding among rodents that are naturally infected by *C. hepatica* (JEONG et al., 2008; SINGLA et al., 2013; MOREIRA et al., 2013). Microscopically,

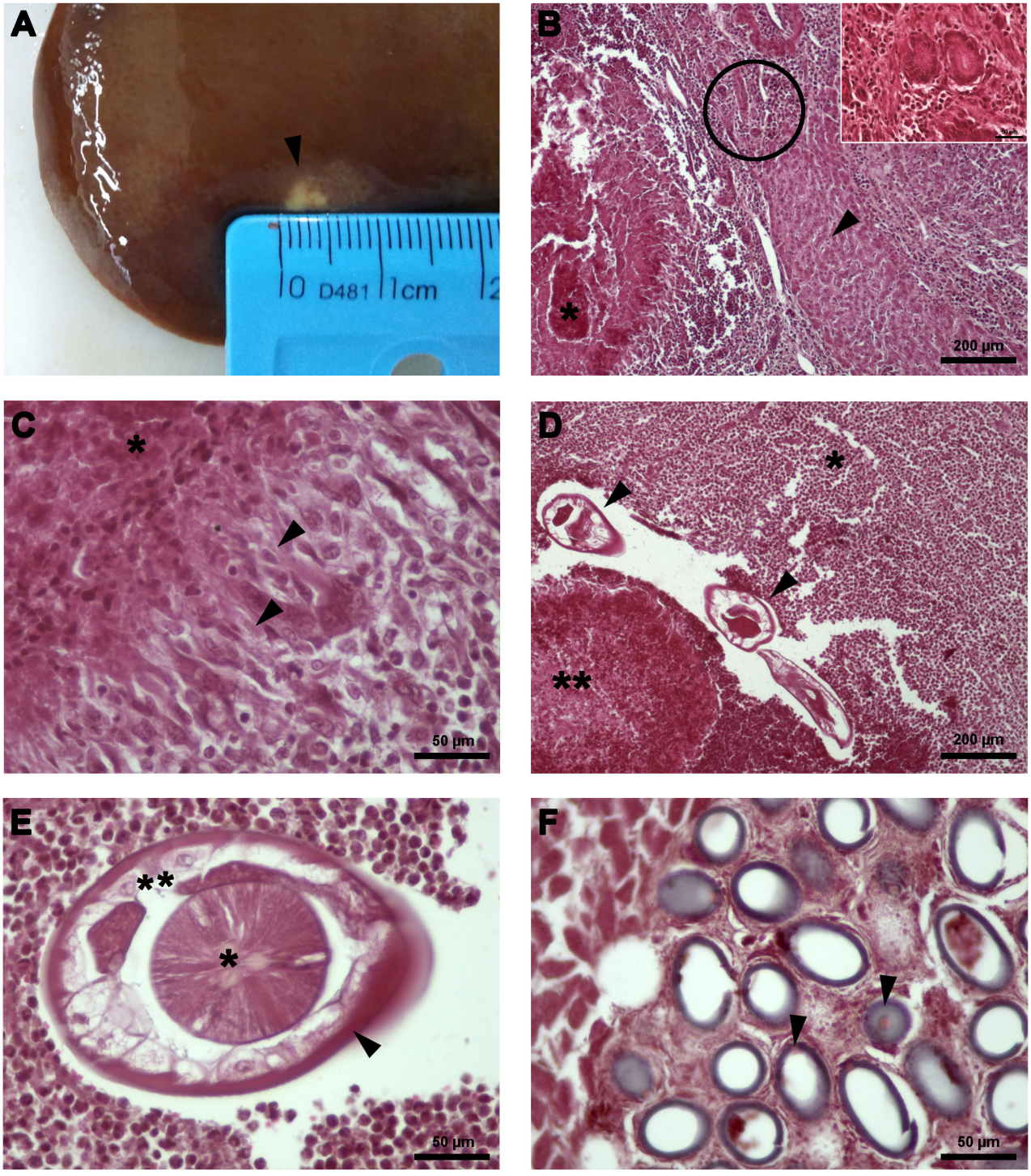

Figure 2. Liver of *Ovis aries* infected by *Capillaria* sp. (A) Macroscopic lesion (granuloma) (arrow); (B) Hepatic parenchyma with calcified granuloma (*), ductal proliferation (circle and insite) and pseudolobules (arrow) (H&E, 100X); (C) Necrosis (*) and palisade cells (arrows) (H&E, 400X); (D) Hepatic parenchyma with leukocyte infiltration (*), coagulative necrosis (**) and a section through a nematode helminth (arrow) (H&E, 100X); (E) Detail of the helminth showing the muscle layer of the body wall (arrow), triradiate pharynx (*) and pseudocoelom (**) (picrosirius, 400X); (F) Cluster of *Capillaria* sp. eggs (arrow) (H&E, 400X).

the alterations to the hepatic parenchyma of rodents range from inflammatory lesions to fibrotic lesions (ANDRADE & ANDRADE, 2004; JEONG et al., 2008; GABAN et al., 2010). Moreover, nematode fragments and clusters of *C. hepatica* eggs have also been observed, which are common findings in rodent infections (MOREIRA et al., 2013). Likewise, sections from bovine hepatic tissue have revealed possible infection by *Capillaria* sp. (NAKAMURA, 2005).

Capillarids are known to present complex taxonomy because of their wide diversity of hosts, including rodents (FUEHRER, 2014a;

SIMÕES et al., 2014; WALKER et al., 2017) and other mammals (FUEHRER, 2014b). Occurrences of *C. bovis* have been reported in the gastrointestinal tract of ruminants (JUSTINE & FERTÉ, 1988, 1989; DIES & COUPLAND, 2001; BOLUKBAS et al., 2012; DAVIDSON et al., 2014; MACIEL, 2014). In contrast to these findings, the present study revealed the presence of hepatic infection in two sheep, possibly caused by *C. hepatica* (syn. *Calodium hepaticum*), which presents low host specificity and high liver tropism. There is a report of *Agouti paca* (paca) naturally infected by *C. hepaticum* from the municipality of Bujari (state of Acre) (ALMEIDA et al., 2013). Other studies have also shown infections among synanthropic rodents (*Rattus norvegicus* and *Rattus rattus*) in Belém, in the eastern Amazon region (MOREIRA et al., 2013).

The ecology of capillariasis should be highlighted. Both adult worms and unembryonated eggs are located in the liver of definitive hosts, from which they can not be disposed. However, the parasite evolved two transmission pathways which indirectly overcome this barrier. First, decomposed liver from dead hosts liberate eggs in the environment. The other strategy includes predators, scavengers and cannibals animals, which release unembryonated eggs in the feces. Because some capillarids are soil-transmitted helminth, the life cycle progresses when unembryonated eggs find adequate conditions for development (soil texture, pH and temperature) and eggs become embryonated or infective (GONÇALVES et al., 2012). It is likely that the ground was contaminated with eggs released from synanthropic and wild rodents (MOREIRA et al., 2013; ALMEIDA et al., 2013). Although liver capillariasis due to *C. hepaticum* in ovine has never been previous reported in Brazil, the inspection ovine meat for human consumption should be carried out to avoid the potential risk for humans (UNGAR et al., 1990; TESSELE et al., 2013). In addition, other studies are required to evaluate a possible impact on the sheep production.

Conclusion

Thus, this first report of *Capillaria* sp. among sheep in the state of Acre is of great importance from a public health point of view. It indicates that there is a need for the Institute of Agricultural and Forestry Sanitary Protection (IDAF) to develop preventive actions in relation to the current management strategies for sheep farming in this state, so as to avoid increased occurrences of this parasitosis among herds in the state of Acre and also contamination among human populations.

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