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CASE REPORT
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Focal Nodular Hyperplasia of the Liver in a Free-Ranging Opossum (*Didelphis marsupialis*)

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ABSTRACT

***Background*:** Focal Nodular Hyperplasia (FNH) in the liver is a solid lesion characterized by spontaneous benign non-neoplastic hepatocellular proliferation that occurs in animals and humans. The clinical course of FNH is usually asymptomatic in animals and humans; and its diagnosis is often an incidental finding during surgery, necropsy/autopsy, or imaging procedures for unrelated symptoms. Despite the lack of clinical significance or malignant transformation potential, FNH is an important differential diagnosis of hepatocellular adenoma, well-differentiated hepatocellular carcinoma, nodular regenerative hyperplasia and metastatic disease. FNH is particularly uncommon in wild marsupials and other wild animals and this study aimed to describe for the first time the occurrence of hepatic FNH in a free-ranging marsupial.

***Case*:** During the fieldwork activities for investigation of liver diseases in wild animals, a well-demarcated, non-encapsulated focal nodule measuring 2.0 x 1.5 x 2.0 cm was observed on the parietal surface of right liver lobe of a wildlife adult female opossum (*Didelphis marsupialis*) from Anajás City, Marajó Island, Brazil. The nodule had a smooth, tan-to-yellow cut surface with small reddish areas devoid of visible fibrous bands and/or scars. The opossum was in good body condition and no other gross lesions were observed. Liver samples were collected, fixed in 10% buffered formalin and routinely processed for histopathological analysis. Tissue samples were embedded in paraffin, cut in 5 μm sections, stained with hematoxylin and eosin (HE), Masson's trichrome (MT), reticulin and Perls stains and the slides were evaluated under light microscope. Liver tissue samples were also snap frozen in liquid nitrogen and submitted to DNA and RNA extraction for molecular screening for *Orthohepadnavirus* and *Hepacivirus* (homologous to hepatitis B and C viruses respectively) by previously described nested PCR and RT-PCR techniques. Light microscopy revealed a well-demarcated, non-encapsulated nodule discernable by compression of the adjacent liver parenchyma. Hepatocytes in this nodule had a central nucleus and cytoplasmic vacuolation with moderate micro and macrogoticular steatosis; FNH lesions presented with discrete mononuclear infiltrate and absence of necrosis, cellular atypia and/or mitotic cells. Hepatocytes were arranged in simple or double layers and there were portal tracts and central hepatic veins**.** Portal and perivascular fibrosis and few foci of mild ductal hyperplasia were evident in MT stain. The surrounding liver parenchyma was free from inflammation, degeneration and/or fibrosis. Liver samples were negative for *Orthohepadnavirus* and *Hepacivirus* infections. Based on gross and microscopic findings, the morphologic diagnosis was FNH.

***Discussion*:** This article provides the first description of hepatic focal nodular hyperplasia (FNH) in a free-ranging opossum (Didelphidae: *Didelphis marsupialis*) living in the Eastern Brazilian Amazon region and it was compared with FNH in humans and other animal species. Liver samples were submitted to gross and microscopic evaluation and tested for *Orthohepadnavirus* and *Hepacivirus* infection. The diagnosis of FNH in a free-living opossum contributes to comparative pathology of the liver and emphasizes the need for further investigation of potential etiologies of this condition in wild animals.

Keywords: liver, nodule, Opossum, hepatotropic virus.

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INTRODUCTION

Focal Nodular Hyperplasia (FNH) of the liver is a solid lesion affecting animals and humans and characterized by spontaneous, benign, non-neoplastic hepatocellular proliferation [6]. Among animals, FNH is more common in dogs aged over 8 years regardless of breed or gender [5,9,10,12,35]. FNH lesions may have focal or multifocal distribution and are macroscopically similar to hepatic adenomas [9,10]. FNH of the liver has been less frequently described in captive nonhuman primates [13,30], laboratory rodents [2,20,34], cats [9], pigs [17] and marsupials [19].

FNH is usually asymptomatic and often an incidental finding during unrelated surgical or imaging procedures, or at necropsy/autopsy [3,9,10,23,30,35,36]. Despite the lack of clinical significance or malignant transformation potential, FNH is an important differential diagnosis of hepatocellular adenoma, well-differentiated hepatocellular carcinoma, nodular regenerative hyperplasia and metastatic disease [3,9,10,29,35].

Marsupials of the *Didelphis* genus are widely distributed in the Americas. Alongside *D. albiventris, D. aurita* and *D. imperfecta*, the common opossum *D. marsupialis* is one of 4 opossum species found in Brazil [8,24].

FNH can generally be diagnosed without immunohistochemistry; however, differential diagnosis between FNH and hepatic adenoma can sometimes be challenging and call for immunohistochemical panels [22].

This study describes pathological features of FNH of the liver diagnosed in a free-ranging *Didelphis marsupialis* captured in Marajó island, Pará, Brazil, and results of molecular screening for *Orthohepadnavirus* and *Hepacivirus*.

CASE

An adult female opossum (*D. marsupialis*) was used in this study. The animal had been captured and slaughtered for human consumption by a local native hunter in the city of Anajás, Marajó island, Brazil, and was recovered during the fieldwork phase of a research project investigating liver diseases in wild animals in 2013 (license number: SISBIO 006/2007).

Gross evaluation revealed a well-demarcated, non-encapsulated focal nodule measuring 2.0 x 1.5 x 2.0 cm on the parietal surface of right liver lobe (Figure 1). The nodule had a smooth, tan-to-yellow cut surface with small reddish areas devoid of visible fibrous bands and/or scars. The opossum was in good body condition and no other gross lesions were observed.

Liver samples were collected, fixed in 10% buffered formalin and routinely processed for microscopical analysis. Tissue samples were embedded in paraffin, cut in 5-µm sections and stained with hematoxylin and eosin (HE), Masson's trichrome (MT), reticulin and Perls stains. Slides were evaluated under light microscope (Eclipse Ni-U)[1] coupled to a digital camera (DS-U3)[1].

Liver tissue samples were snap frozen in liquid nitrogen and submitted to DNA and RNA extraction using commercial kits DNeasy Blood and Tissue[2] and RNeasy mini kit[2] respectively. Extracted DNA and RNA were used for molecular screening for *Orthohepadnavirus* and *Hepacivirus* (homologous to hepatitis B and

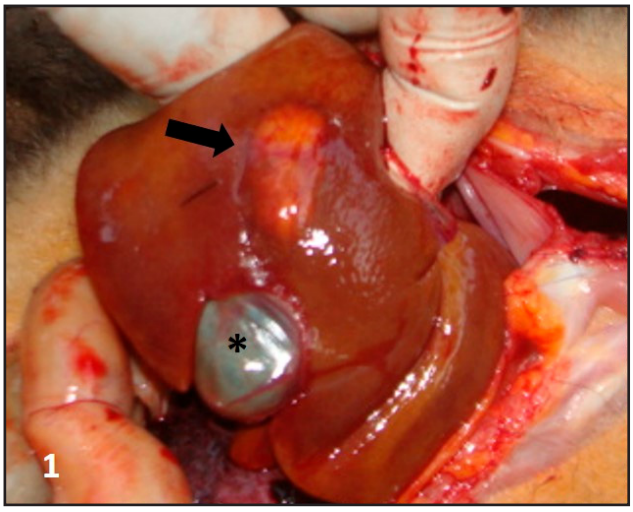

Figure 1. Focal nodular hyperplasia (FNH) affecting the parietal surface of the liver (arrow), next to the gallbladder (*) of an adult female common opossum (*Didelphis marsupialis*) captured in Marajó island, Pará, Brazil.

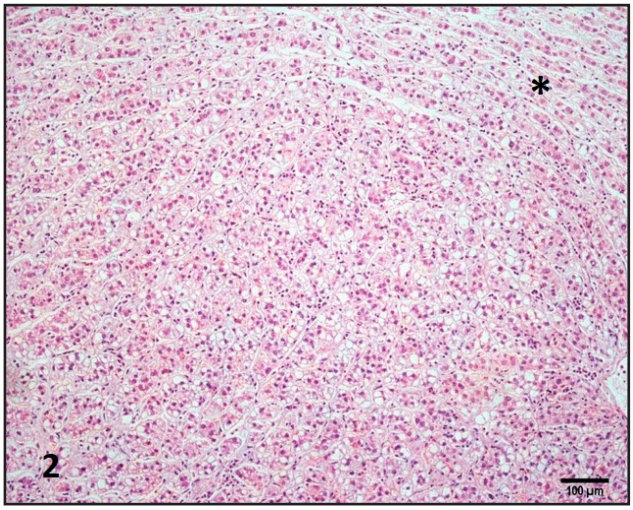

Figure 2. FNH causing mild compression of surrounding liver parenchyma (*) [HE, 10x magnification].

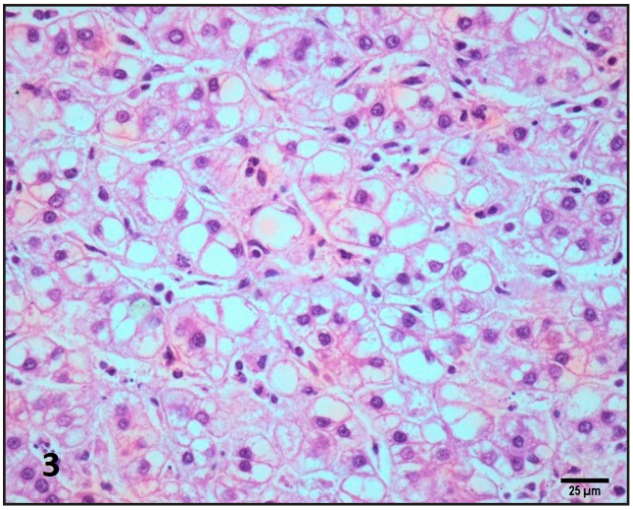

Figure 3. FNH, vacuolated hepatocytes arranged in double layers [HE, 40x magnification].

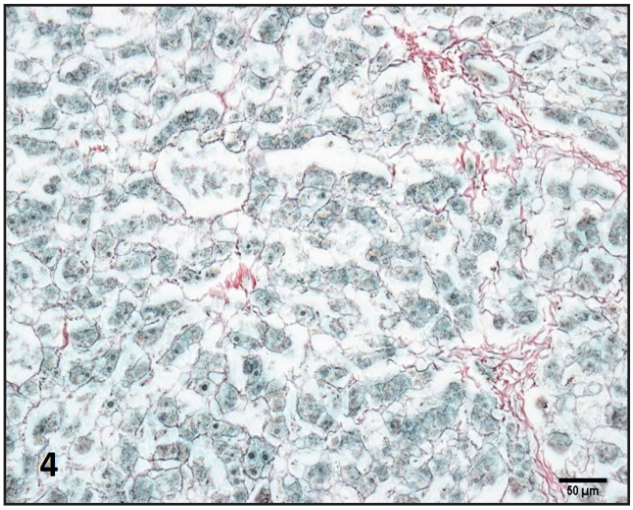

Figure 4. FNH, hepatocytes arranged in double layers, preserved reticulin framework and fibrosis [Reticulin, 20x magnification].

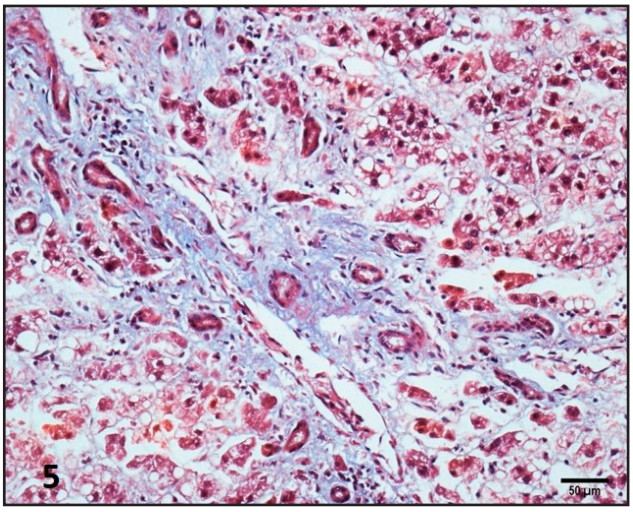

Figure 5. FNH, portal fibrosis, ductal hyperplasia and vacuolated hepatocytes in hepatic lobules [MT, 20x magnification].

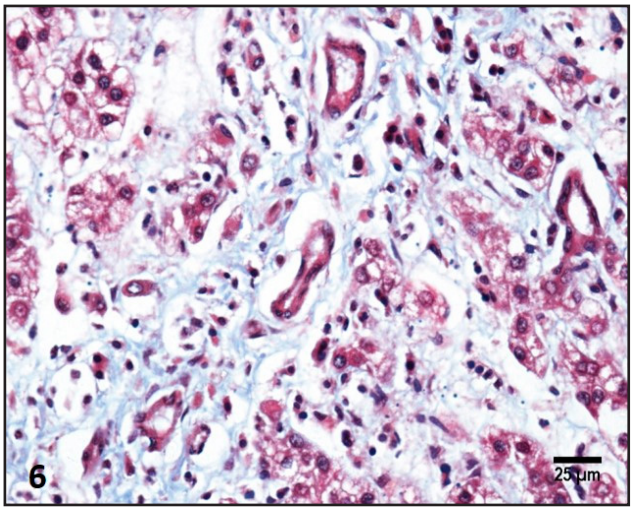

Figure 6. FNH, lobular fibrosis and ductal hyperplasia [MT, 40x magnification].

C viruses respectively) by previously described nested PCR and RT-PCR techniques, respectively [11,25].

Light microscopy revealed a well-demarcated, non-encapsulated nodule discernable by compression of the adjacent liver parenchyma (Figure 2). Hepatocytes in this nodule had a central nucleus and cytoplasmic vacuolation with moderate micro and macrogoticular steatosis; FNH lesions presented with discrete mononuclear infiltrate and absence of necrosis, cellular atypia and/or mitotic cells mitotic cells (Figure 3). Hepatocytes were arranged in simple or double layers (Figure 4) and there were portal tracts and central hepatic veins**.** Portal and perivascular fibrosis (Figure 5) and few foci of mild ductal hyperplasia (Figure 6) were evident in MT stained slides. The surrounding liver parenchyma was free from inflammation, degeneration and/or fibrosis. Liver samples submitted to nested PCR and RT-PCR protocols were negative for *Orthohepadnavirus* and *Hepacivirus* infections. Based on gross and microscopic findings, the morphologic diagnosis was FNH.

DISCUSSION

This is the first description of hepatic FNH in a free-ranging opossum and is in agreement with previous reports of similar lesions in other species, including humans [6,9,10,13]. Gross findings resembled typical FNH lesions described in animals and humans: single, solid, non-encapsulated or partially encapsulated nodules with lobulated appearance and well-defined margins, and lighter than surrounding liver tissue [3,9,10, 13,16,17,29,32]. FNH lesions found in the liver of the common opossum analyzed in this study were also consistent with previous descriptions of FNH in chimpanzees (*Pan troglodytes*) [30], cynomolgus

monkeys (*Macaca fascicularis*) [13], dogs [9,10,35] and rats [34].

FNH of the liver has similar morphological characteristics in animals and humans [5,9,10,12,13,30,35] and the diagnosis is based on microscopic findings such as non-encapsulated focal/multifocal lesions consisting of often vacuolated, well-differentiated hepatocytes arranged in simple or double layers, which compress the surrounding normal liver tissue [9,10,34,35].

Although uncommon, the following nodular hepatic lesions have been described in captive marsupials: hepatic vascular proliferation in common wallaroo (*Macropus robustus*) [7], lymphoma in fat-tailed false antechinus (*Pseudantechinus macdonnellensis*, previously classified as *Antechinus macdonnellensis*) [1], cholangiocarcinoma in koala (*Phascolarctos cinereus*) [7] and hepatic adenoma (previously designated as hepatoma) in pygmy possums *Cercartetus nanus* [7], *Cercartetus concinnus* [18] and in Tasmanian devils (*Sarcophilus harrisii*) [15]. FNH of the liver has also been described in captive gray short-tailed opossum (*Monodelphis domestica*) [19]. Additionally, FNH is particularly uncommon in wild marsupials and other wild animals [9,10].

In the case described, differentiation between FNH of the liver and hepatocellular adenoma was based on common microscopic characteristics of FNH in humans and animals: normal lobular architecture, hepatocytes arranged in double layers, preserved reticulin framework, presence of portal tracts and central hepatic veins in the lesion and lack of cellular atypia and/or mitosis [9,10]. Classical histologic features of chronic hepatitis, such as lobular and/or portal inflammation, necrosis and/or fibrosis, were lacking; hence, nodular regenerative hyperplasia also was excluded [9,10]. In the opossum in this study, changes such as periportal and lobular fibrosis were limited to the nodule and did not affect surrounding tissues.

Hepatic fibrosis/cirrhosis, nodular regenerative hyperplasia and neoplastic lesions may be observed in human patients with chronic hepatitis caused by hepatitis B and C viruses [22]. Therefore, liver samples in this study were submitted to molecular screening for HBV-like and HCV-like viruses, despite the absence of microscopic findings consistent with chronic hepatitis. Negative results confirmed the absence of these two hepatotropic viruses in the opossum liver and ruled out these agents as cause of FNH.

The etiology of human FNH is not completely understood. The pathogenesis of the disease was first attributed to use of oral contraceptives, but recent studies have refuted this hypothesis [4,21,26,27]. The pathogenesis of FNH in animals remains to be determined [9,10]. FNH was found not to be associated with the use of steroids or extrahepatic disease in dogs [5]. Exposure to chemical compounds has been related to proliferative hepatic lesions (including FNH) in rats; however, such lesions are uncommon and a direct association between FNH development and exposure to chemical compounds has not been established [2,34,16]. In this study, it could not be determined whether environmental exposure to chemical compounds played a role in the occurrence of FNH; still, this hypothesis is worthy of consideration in future investigations of FNH in free-ranging opossums.

Recently, the etiology of human FNH has been associated with congenital or acquired intrahepatic or systemic vascular anomalies leading to localized arterial hyperperfusion of the hepatic parenchyma and ultimately to focal hepatocyte proliferation [14,23,31]. In humans, the presence of unusually large vessels in FNH lesions has been related to nonspecific responses to focally increased blood flow [3]. With the exception of ductal hyperplasia, portal and lobular fibrosis in FNH lesions, vascular changes were not observed in the hepatic parenchyma in this study. Central fibrous scars are another commonly described feature of FNH in humans [28,29,31,33] which is thought to be related to activation of hepatic stellate cells and increased expression of vascular endothelial growth factor (VEGF) [31]. These lesions were also absent in the nodule described.

Spontaneous development of FNH lesions in the liver of a free-ranging *Didelphis marsupialis* described here emphasizes the importance of further investigations of the pathogenesis of hepatic FNH in animals. Comparative environmental pathology studies are warranted to determine the etiology of FNH and to identify other wild species potentially affected by this condition.

This report is intended to increase the current understanding of FNH and comparative pathology of liver, with potential contributions to the differential diagnosis of liver diseases in wild animals.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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