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SHORT COMMUNICATION

# Hemochromatosis-like disease in Brazilian tapirs (*Tapirus terrestris*) in Pará state, Brazil

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## ABSTRACT

We report two cases of hemochromatosis-like disease in captive Brazilian tapirs, *Tapirus terrestris* in Pará state, Brazil. Both animals presented symptoms of chronic hepatopathy associated with marked accumulation of hemosiderin. The coloration of Perls demonstrated pronounced iron accumulation in macrophages in the portal space, Kupffer cells, and, to a lesser extent, in the hepatocytes of the periportal region. Marked portal fibrosis was evidenced by Masson's trichrome. The pathological mechanisms of this disease in tapirs are not yet well established. It has been suggested that the species may have different mechanisms of iron absorption and elimination, rendering them sensitive to elevation in dietary levels of this metal. Two previous reports of this disease in *T. terrestris* exist from zoos in Australia and Scotland. This is the first report of this disease in tapirs in Brazil based on histopathological and histochemical findings.

**KEYWORDS:** hepatopathy, hemosiderin, Perissodactyla, Amazonia

# Hemocromatose símile em antas brasileiras (*Tapirus terrestris*) no estado do Pará, Brasil

## RESUMO

Relatamos dois casos de hemocromatose símile em antas brasileiras, *Tapirus terrestris* mantidas em cativeiro no estado do Pará, Brasil. Ambas apresentavam lesões de hepatopatia crônica e acentuada, associadas a acúmulo acentuado de hemossiderina. A coloração de Perls demonstrou acúmulo acentuado de ferro nos macrófagos do espaço porta, células de Kupffer e, em menor extensão, hepatócitos na região periportal. Havia, ainda, fibrose portal marcada evidenciada por tricrômico de Masson. Os mecanismos patológicos da doença em antas ainda não estão bem estabelecidos e tem sido sugerido que a espécie apresenta diferentes mecanismos de absorção e eliminação de ferro, sendo sensíveis à elevação dos níveis dietéticos desse metal. Existem dois relatos anteriores da doença em *T. terrestres* em zoológicos na Austrália e na Escócia. Este é o primeiro relato da doença em antas no Brasil.

**PALAVRAS-CHAVE:** hepatopatia, hemossiderina, Perissodactyla, Amazônia

Hemochromatosis is a disease diagnosed in several animal species, including birds, tapirs, bats, dolphins, pinnipeds, rodents, rhinoceros, lagomorphs, human and nonhuman primates (Clauss and Paglia 2012). Hereditary factors due to mutations in genes that regulate iron homeostasis may contribute to this disease (Keeble and Scudamore 2001; Clauss and Paglia 2012; Siddique and Kkowdley 2012). In humans, the genetic basis of the disease is well established (Cançado *et al.* 2010), but it is not yet well understood in other animals. The first cases of tapir hemochromatosis reported in the literature occurred in *Tapirus terrestris* (Linnaeus, 1758) (Keeble and Scudamore 2001; Peters *et al.* 2012) and *T. bairdii* (Gill, 1865) (Bonar *et al.* 2006).

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Hemosiderosis and hemochromatosis are terms used to indicate excess iron (Fe) in the body, which is characterized by accumulation in tissues of a golden-brown granular pigment called hemosiderin. Hemosiderin accumulation in a tissue or organ without causing functional damage is called hemosiderosis. Hemochromatosis, on the other hand, is characterized by abnormal iron deposition, causing tissue dysfunction that is most pronounced in the liver parenchyma (Brown *et al.* 2017). Excessive deposition of Fe in tissues may occur due to high levels of this element in the diet, genetic metabolic disorders, hemolytic diseases (Grotto 2008), or nutritional copper deficiency (Tokarnia *et al.* 2010). Another important factor in Fe metabolism is the expression of hepcidin, which has been demonstrated in recent years to be a key protein in preventing hemochromatosis, as it plays a fundamental role in Fe homeostasis by regulating levels of the cellular iron exporter ferroportin (Grotto 2008; Pantopoulos 2018).

Tapirs, together with rhinoceros and equidae, are ungulate mammals belonging to the order Perissodactyla. This close taxonomic relationship has allowed application of the same strategy to the diagnosis of some diseases. Reports of iron (Fe) metabolism disorders in horses and rhinos (Pearson *et al.* 1994; Pearson and Andreasen 2001; Clauss and Paglia 2012) led to the investigation and diagnosis of these same disorders in tapirs (Pearson *et al.* 1994; Bonar *et al.* 2006).

Here we report two cases of a hemochromatosis-like disease in Brazilian tapirs (*Tapirus terrestris*) and provide histological and histochemical chracterization of the condition in tissues of the animals.

The two Brazilian tapirs (tapirs 1 and 2) were housed at Fundação Zoobotânica (5°24'20"S, 49°3'32"W) in Marabá city, Pará state, Brazil. Tapir 1 was a male and about 15 years old when it died. No additional information on the animal's history or necropsy findings was provided. Tapir 2 was a female, about 20 years old and died after vocalizing distress in a lateral decubitus position with extended limbs. The diet of the animals consisted of corn, horse feed, bovine mineral salt, bananas, grass, and fruits (mainly mangoes and genipaps). The animals were necropsied on site and tissue samples were removed (liver, spleen, tongue, heart, kidney, and bladder from tapir 1, and liver, spleen, tongue, heart, kidney, intestine, and stomach from tapir 2). The samples were stored in formaldehyde and sent to the Institute of Veterinary Science at Universidade Federal do Pará (Castanhal, Pará state, Brazil) in January 2015 (tapir 1) and February 2017 (tapir 2) for histopathological diagnosis.

Tissue fragments were routinely processed for histopathological examination, embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin and observed under an optical microscope (Nikon Eclipse 50i). Selected liver sections were stained with Perls and Masson's trichrome (De Tolosa *et al.* 2003).

Under the microscope, the main lesion apparent in both animals was seen in the liver. In this organ, a lumpy brownish pigment (hemosiderin) was observed in large amounts in the cytoplasm of Kupffer cells, and in macrophages agglomerated in the portal spaces (Figure 1a,c) and among the hepatocyte cords (only in tapir 2). The same pigment was found in hepatocyte cytoplasm in mild to moderate amounts, mainly in the periportal zone (only in tapir 1) (Figure 1a). The same areas containing brownish pigment also stained in blue when exposed to Perls (Prussian blue reaction) in the same intensity and distribution (Figure 1b). Both animals also had portal fibrosis, which varied from mild to moderate in tapir 1, and from moderate to severe in tapir 2. This fibrosis was evidenced by Masson's trichrome staining, which made it possible to observe an excess of collagen fibers in the hepatic portal space and in the hepatocyte cords (Figure 1d). Other hepatic findings in tapir 1 included moderate diffuse hepatic congestion, and a caseocalcareous granuloma, characterized by a central area of caseous necrosis, strongly mineralized and bordered by a thick fibrous capsule. The spleens of both tapirs showed moderate accumulation of lumpy brown pigment (hemosiderin) in the cytoplasm of macrophages in red pulp. The spleen of tapir 1 was bloodless and had moderate lymphoid depletion in the white pulp follicles.

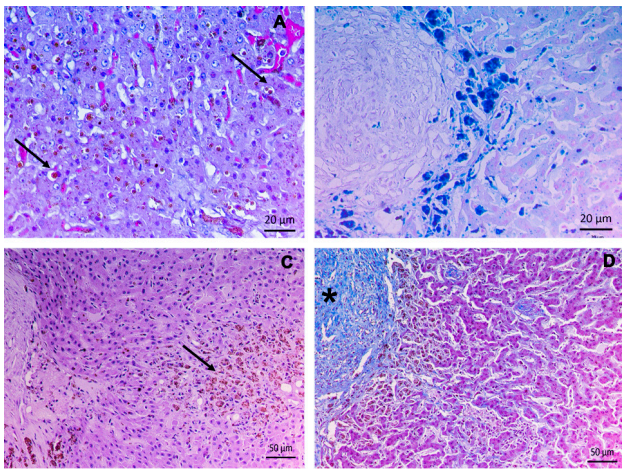


**Figura 1.** Histological and histochemical characterization of a hemochromatosis-like disease in the liver of Brazilian tapirs (*Tapirus terrestris*). A – A lumpy brown pigment in the cytoplasm of hepatocytes, Kupffer cells and macrophages (black arrows), stained with hematoxylin & eosin (HE). 40x magnification; B – Blue staining observed mainly in the cytoplasm of macrophages in the portal space (black arrows), and, to a lesser extent, in Kupffer cells and hepatocytes, indicating the presence of iron in the accumulated brownish pigment. Perl's staining, 40x; C – A large amount of brownish pigment in the cytoplasm of Kupffer cells and macrophages clustered in the portal space or found randomly in the liver parenchyma (black arrow) and portal fibrosis. HE. 20x; D – Portal fibrosis evidenced by Masson's trichrome staining (asterisk). 20x.This figure is in color in the electronic version.

In both tapirs, discrete foci of bronchopneumonia were observed, characterized by infiltration by small numbers of neutrophils and macrophages. Tapir 1 also presented intralesional plant fibers and moderate diffuse pulmonary congestion. The tongue fragment from tapir 1 presented moderate orthokeratotic hyperkeratosis, with yeast-like structures in the superficial layers of the stratum corneum. The tissue samples of the heart and kidney of both tapirs, the bladder of tapir 1, and the intestine and stomach of tapir 2 did not present lesions worthy of note.

Based on the similarity of our histopathological and histochemical findings with those described in previous studies on tapirs (Pearson *et al.* 1994; Bonar *et al.* 2006; Peters *et al.* 2012) and other animal species (Pearson and Andreasen 2001), we diagnosed the two examined tapirs with a hemochromatosis-like disease. The liver lesions identified by microscopy were characteristic of chronic liver disease, displaying varying intensities of portal fibrosis, highlighted by Masson's trichrome, and accumulation of hemosiderin, mainly in macrophages, as evidenced by Perls stain. These injuries were identical to those described by Peters *et al.* (2012) in Brazilian tapirs in captivity in Australia.

Our results agree with previous reports in that hemochromatosis or hemochromatosis-like disease in tapirs may be associated with severe clinical symptoms of liver insufficiency (Pearson *et al.* 1994). Since it was first described, within the past decade, few studies have been published on the disease in captive tapirs. In these cases, as in our study, the causes of the disease were not unequivocally determined. However, it is suggested that factors related to human care for tapirs in captivity may contribute to the development of the disease (Pearson *et al.* 1994).

An important factor to consider in cases of hemochromatosis is nutrition. The diet of affected tapirs was reportedly supplemented with horse feed and a mineral mixture for cattle, that commonly contains iron (Andriguetto 2002). Bonar *et al.* (2006) and Peters *et al.* (2012) hypothesize that tapirs are predisposed to hemochromatosis due to an extremely efficient dietary iron absorption compared to other animal species, or that they may have a slower iron excretion rate than other species due to low levels of this metal in their natural habitats. As occurs in birds (Mete *et al.* 2003), these authors suggest that, when tapirs have access to high levels of iron, as in captive conditions, they are unable to regulate this compensatory mechanism and thus face increased risk of hemochromatosis (Pearson *et al.* 1994; Bonar *et al.* 2006). A similar condition may have afflicted the tapirs in the present study.

Another possible source of iron that must be considered is the water to which the animals have access. Studies of rivers in the northern region of Brazil have demonstrated that they carry high levels of iron (Miranda *et al.* 2009), above those listed as adequate in the resolution of the Brazilian National Environment Council (CONAMA 2005). These high values are explained by the geochemical composition of bedrocks and substrates in the region (Miranda *et al.* 2009). Thus, the iron contained in the water may also have contributed to the development of the clinical and pathological condition of the animals in our study.

Based on a study involving oral administration of excess iron to adult ponies, Pearson and Andreasen (2001) reported that it was difficult to induce hemochromatosis by administration of free iron in food or water. The authors suggest that the existence of a previous liver injury or concomitant hemosiderosis is necessary for the condition to develop. In the present case, no other agents or injuries that could underlie liver disease were observed. Although there was moderate splenic hemosiderosis in both tapirs, there were no other splenic, hepatic, or renal lesions indicative of hemolytic disease. Hemolytic diseases are not normally associated with hepatic fibrosis (Cullen and Stalker 2016).

Here we demonstrate that a hemochromatosis-like disease, possibly hemochromatosis, occurs in captive Brazilian tapirs in the Amazon region, and that this disease can lead to severe clinical conditions and liver failure. The elucidation of the pathological and genetic mechanisms of the disease in tapirs require additional studies, but captive animals should be monitored, and their dietary intake of iron should be controlled.

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