



Case Report

Lafora's disease in a free-ranging toco toucan (*Ramphastos toco*) with neurologic disease

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Abstract

Lafora's disease is a genetic disease associated to mutations in genes that encodes laforin and malin, which results in intracellular polyglucan storage. The present report describes a case of Lafora's disease in a toco toucan with episodes of incoordination and myoclonus that resulted in traumatic lesions and fracture of the left hindlimb. The bird was euthanized and submitted to necropsy. Microscopically there were abundant PAS-positive and diastasis-resistant Lafora's inclusion bodies in neurons in the cerebellum, supporting the diagnosis of Lafora's disease.

Keywords: neurodegenerative disease, neurodegeneration, wild bird, Lafora body.

Introduction

Lafora's disease (LD) is a rare autosomal recessive and fatal disease associated with severe myoclonic epilepsy due to intracellular polyglucan accumulation. In humans, LD is associated with recessive mutations in two epilepsy progressive myoclonus (EPM) genes, namely *EPM2A* and *EPM2B* (the later also known as *NHLRC1*), which encodes laforin, a carbohydrate-bidding phosphatase, and malin, an E3 ubiquitin ligase 1, respectively (12, 15). With a non-functional laforin and malin complex, abnormal glycogen becomes insoluble and, consequently, there is accumulation of glycogen and glycosaminoglycans inside the cells, which is microscopically observed as polyglucosan inclusion bodies (12). This abnormal accumulation induces neuronal dysfunction, which may result in neurological signs (15).

In human patients, clinical manifestation starts in adolescence with epileptic episodes that progress to myoclonic seizures, neurodegeneration, and sometimes with vegetative

state before death (13). The diagnostic hallmark of the disease is the presence of Lafora's inclusion bodies, which can be observed in the brain, heart, skin, skeletal muscle, and liver (13). Recently, it has been demonstrated that Lafora's inclusion bodies in the brain may affect not just neurons, but also astrocytes (3).

Although less frequently than in humans, LD have also been reported in domestic and wild animals, with many reports in dogs (2, 4, 14, 16). In dogs, LD has been associated with a mutation in the *EPM2B* gene, which consists of a massive expansion of the dodecamer repeat sequence, which has been exclusively described in dogs (2, 10, 14). The most common clinical signs in affected dogs include reflex or spontaneous myoclonic events, seizures, and jaw smacking (14, 17).

There are fewer reports of LD in animal species other than dogs, including cow (*Bos taurus*), cat (*Felis catus*), gray-headed flying fox mega bat (*Pteropus poliocephalus*), cockatiel (*Nymphicus hollandicus*), and fennec fox (*Vulpes*

zerda) (1, 5, 6, 8, 9, 11). However, in these other species no genetic mutation associated to LD has yet been investigated. Additionally, there is one report of Lafora bodies in a racoon without association with neurological signs (7). The present report describes a case of LD in a wild toco toucan (*Ramphastos toco*) with neurological disease.

Case Description

A male free-ranging juvenile toco toucan (*Ramphastos toco*) was admitted at the Municipal Zoo Park in Bauru (State of São Paulo, Brazil), for veterinary assistance with a long laceration on the left wing. The lesion was cleaned, sutured, and treated with enrofloxacin (15 mg/kg, IM SID) tramadol (20 mg/kg, IM SID) and meloxicam (1 mg/kg, IM SID) for 10 days. Four months later, the bird developed incoordination, and the humerus-radio-ulnar joint at the left wing was swollen, with multiple dark red areas. A presumptive diagnosis of joint inflammation was made, and fluid therapy associated to intramuscular enrofloxacin (30 mg/kg IM SID) and meloxicam (0.5 mg/kg IM SID) was initiated. Four days later, despite the treatment, the bird remained severely uncoordinated with myoclonic episodes that resulted in a fracture of the left tibiotarsus. Due to the poor prognosis the bird was euthanized and immediately subjected to necropsy.

Grossly, the humerus-radio-ulnar joint was swollen with moderate multifocal cutaneous hemorrhages and there was a fracture of the left tibiotarsus. No other gross lesions were observed. Samples of the lungs, heart, brain, liver, kidneys, intestines, proventriculus, ventriculus, adrenal, pancreas, esophagus, trachea, and the affected joint were fixed in 10% buffered formalin and processed for paraffin-embedding, sectioned in a microtome (3 µm-thick sections), and stained with hematoxylin and eosin (HE) and Periodic Acid Schiff (PAS). Resistance to diastase digestion before PAS stain was also evaluated.

Microscopically, the most important lesion was observed in the cerebellum. There were multiple 10-15 µm in diameter spherical structures, with an amphophilic center and a dense basophilic border in neuronal bodies of Purkinje cells and presumably in dendrites in the molecular and granular layers (Fig. 1). These structures were strongly PAS-positive and resistant to diastase digestion, and therefore were considered morphologically and histochemically compatible with Lafora's inclusion bodies (Fig. 2). Additionally, there was a severe multifocal pulmonary edema and there was a marked focally extensive fibrinous and necrotic dermatitis, severe multifocal fibrinous necrotizing and heterophilic synovitis and focally extensive fibrinous necrotic and granulomatous periostitis in the humerus-radio-ulnar joint. Based on the histopathological and histochemical findings as well as the neurological signs observed in this bird, a diagnosis of LD, with cerebellar Lafora's inclusion bodies in the perikaryon and dendrites of neuronal cells, was established.

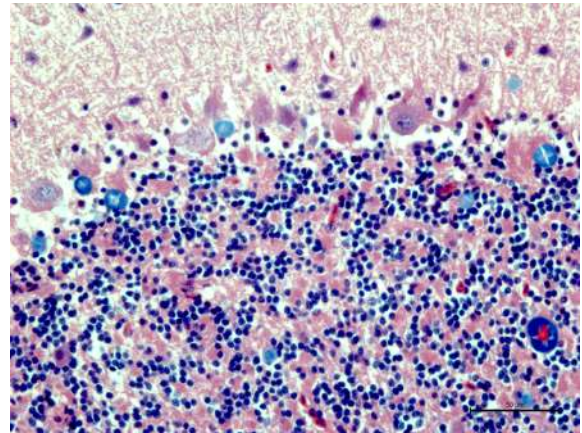


Figure 1. Cerebellum, toco toucan (*Ramphastos toco*). Cerebellum with multiple Lafora's inclusion bodies in Purkinje cells and presumably in dendrites in the molecular and granular layers, with amphophilic center and a dense basophilic border. Hematoxylin and eosin, bar = 50 µm.

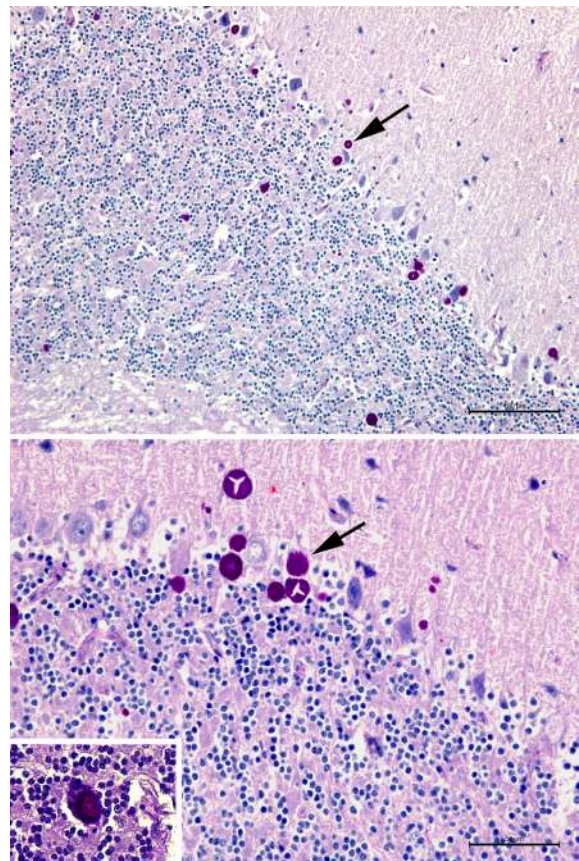


Figure 2. Cerebellum, toco toucan (*Ramphastos toco*). (A) Cerebellum with multiple strongly PAS-positive Lafora's inclusion bodies in Purkinje cells (arrow) and presumably in dendrites in the molecular and granular layers. Periodic Acid Schiff (PAS), bar = 100 µm. (B) PAS-positive inclusion bodies (arrow); inset: PAS-positive and diastasis-resistant Lafora's inclusion body. PAS, bar = 50 µm.

Discussion

This report describes for the first time a case of LD associated with neurological signs in a toco toucan. Neurological disease in this case was characterized by severe incoordination and myoclonus, that resulted in trauma and fracture of the left hindlimb. Histopathological and histochemical findings in this case were compatible with neuronal degeneration due to the polyglucosan accumulation (12). The only reported cases of LD in birds affected two cockatiels (*Nymphicus hollandicus*) one of them with tremors (1), and the other a presumptive case of LD without clinical signs in spite of many PAS-positive and diastase-resistant inclusion bodies in neurons and cardiomyocytes (11).

Neurologic signs associated with LD such as seizures, intermittent head tilt, instable walking, tremors, and unawareness of the environment, have been described in various animal species (6, 8). In this case, incoordination, myoclonus, and postural abnormality were observed.

Lafora's inclusion bodies in this toucan were strongly PAS-positive and diastase-resistant, which are considered diagnostic hallmarks of Lafora's disease in other animal species and humans (1, 5, 6, 8, 9, 14). Although neurons are the cells that more commonly accumulate LD inclusion bodies in animals, these inclusion bodies have also been described in cardiomyocytes and hepatocytes in a fennec fox (8).

In human patients and dogs, it is well established that LD is an inherited condition associated with mutations on the malin and laforin genes. These mutations result in the intracytoplasmic accumulation of polyglucosan, an insoluble form of glycogen (2, 10, 12, 14, 15). Unfortunately, due to the low frequency of cases in birds, there are no studies describing the genetic basis of this disease in these species. This case highlights the importance of *postmortem* evaluation to identify unexpected diseases that can be present in wild birds and reinforce the importance of developing future studies to understand epidemiologic significance of LD in avian species as well as to investigate its pathogenesis in birds.

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Conflict of Interest

The authors declare no competing interests.

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