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Case Report

Inclusion Body Disease in a *Corallus hortulanus*

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Abstract

An adult male Amazon tree boa (*Corallus hortulanus*) from the Zoo-Botanical Foundation (Belo Horizonte, Brazil) died after a period of apathy and anorexia. Cachexia was the only significant gross finding. Numerous eosinophilic intracytoplasmic inclusion bodies were found in the liver, lungs, heart, testes, kidneys, and adipose tissue, compatible with the Inclusion Body Disease. The snake also had an undifferentiated metastatic sarcoma. In order to further characterize the inclusion bodies found in this case, transmission electronic microscopy was performed. Inclusion Body Disease affects boid snakes, causing regurgitation, anorexia and neurological signs that eventually lead to death. This is a challenging disease since its etiology, pathogenesis, and epidemiology are unknown, and therefore treatment is not effective. This is the first report of Inclusion Body Disease in boid snake in Brazil.
Key Words: *Corallus hortulanus*, Inclusion Body Disease, Amazon tree boa, snake, sarcoma.

Introduction

Inclusion Body Disease (IBD) is a fatal disorder that affects boid snakes (members of the families Boidae and Pythonidae). It is characterized by intracytoplasmic inclusion bodies that can be readily observed by light microscopy (2). It was first identified in the United States of America in the 1970s, where the disease affects multiple species of boid snakes in private and zoological collections (9). Viral etiology has been suspected, but the pathogenesis and epidemiology of IBD are unknown, making this disease a significant challenge in reptile collections.

Case report

An adult male Amazon tree boa (*Corallus hortulanus*) housed at the Zoo-Botanical Foundation in Belo Horizonte, Brazil was apathetic and anorexic. The animal died before it could be examined by veterinarians. Necropsy was performed at the zoo and the only recorded gross changes were cachexia and lung congestion.

Fragments of the lungs, liver, heart, testes, kidneys, spleen and adipose tissue were fixed in 10% neutral buffered formalin and sent to the histopathology laboratory at the Universidade Federal de Minas Gerais, Brazil. Tissues were processed for paraffin embedding. Four µm sections were stained with hematoxylin and eosin (HE). Numerous round to oval eosinophilic intracytoplasmic inclusion bodies ranging from 1 to 40 µm in diameter were observed in the liver, lungs, heart, testes, kidneys, and fat tissue. The inclusion bodies had a multifocal to coalescing distribution (Fig. 1-3) and in some areas it was associated with a mild histiocytic inflammatory infiltrate. Inclusions tended to form aggregates in different cell types including hepatocytes (Fig. 1), faveolar epithelium (Fig. 2), cardiomyocytes, testicular interstitial cells (Fig. 3) and seminiferous epithelium, tubular and interstitial renal cells, and adipocytes. There was also a neoplastic proliferation of mesenchymal cells, which was solid, non-encapsulated, poorly demarcated, and highly invasive. Neoplastic cells

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were observed in the testes, spleen, liver (Fig. 1), and lung (Fig. 2).

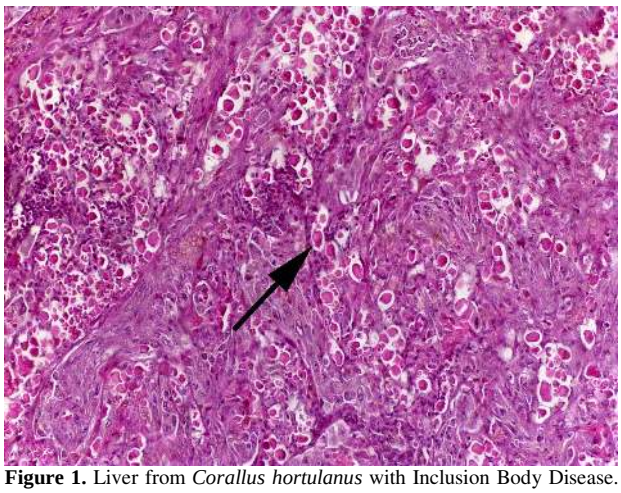


Figure 1. Liver from *Corallus hortulanus* with Inclusion Body Disease. Numerous large intracytoplasmic eosinophilic inclusion bodies (arrow) interspersed with pleomorphic neoplastic cells and diffuse fibrosis. HE, 20x objective.

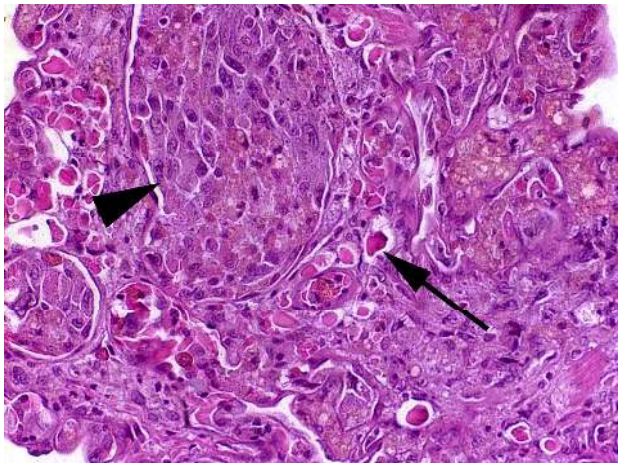


Figure 2. Lung from *Corallus hortulanus* with Inclusion Body Disease. Thickened faveolar wall with numerous large intracytoplasmic eosinophilic multifocal inclusion bodies (arrow). Neoplastic emboli in blood vessels (arrow head). HE, 40x objective.

Neoplastic cells were markedly pleomorphic, with a predominant spindle shape and an abundant eosinophilic cytoplasm. Nuclei were pleomorphic, but predominantly oval with loose chromatin, and one or multiple prominent nucleoli. There was marked anisocytosis and anisokaryosis and high number of mitotic figures. Neoplastic emboli were observed in several blood vessels in the lungs (Fig. 2), liver, and kidney. Other microscopic changes included intense and diffuse macro and micro vacuolization of hepatocytes associated to intense and diffuse fibroplasia and mild multifocal lymphocytic inflammatory infiltrate in the liver. In the testes, there was intense and diffuse fibroplasia associated with intense and diffuse degeneration of the seminiferous tubules. In the kidneys, there was moderate and diffuse fibroplasia, multifocal and moderate thickening of the glomerular membrane with occasional multifocal mineralization, moderate multifocal tubular degeneration, moderate multifocal lympho-plasmacytic inflammatory infiltrate, and moderate multifocal deposition of a light brown cytoplasmic pigment in tubular epithelial cells. In the lungs, there was marked thickening of faveolar walls that was associated with accumulation of neoplastic cells. There was also moderate multifocal fibrosis, mild multifocal lymphocytic inflammatory infiltrate and moderate multifocal macrophages with brown cytoplasmic pigment. These findings were compatible with the IBD (2) associated with a metastatic undifferentiated sarcoma.

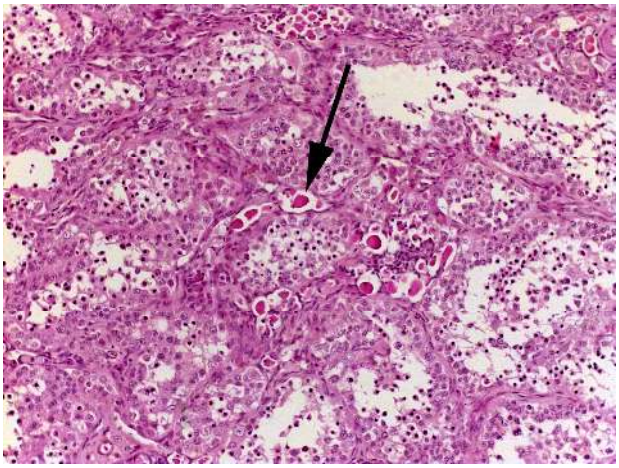


Figure 3. Testis from *Corallus hortulanus* with Inclusion Body Disease. Numerous intracytoplasmic eosinophilic multifocal inclusion bodies in the interstitium (arrow). Moderate degeneration of the seminiferous epithelium. Moderate interstitial fibroplasia and fibrosis. HE, 20x objective.

In order to further characterize the inclusion bodies found in this case, samples of the liver, lungs, testes, and kidneys were submitted to transmission electron microscopy. Samples fixed in 10% neutral buffered formalin were transferred into Karnovsky`s solution in phosphate buffer, washed with 0.1 M phosphate buffer, transferred into 2% osmium tetroxide, washed with saline containing 17.8% sucrose, and then transferred into uranyl acetate aqueous solution with 13.3% sucrose. Samples were then dehydrated through increasing concentrations of ethanol, soaked in acetone, embedded in epon resin, sectioned, stained with lead citrate, and examined under a transmission electron microscope (Tecnai G2-12-120 kV - FEI SpiritBiotwin, USA). Intracytoplasmic homogeneous, solid and electron-dense inclusion bodies ranging from 0.1 to more than 40 µm were observed in several cell types. Smaller inclusions consist of aggregates of electron-dense granular material (Fig. 4).

In order to confirm the histopathological diagnosis of undifferentiated metastatic sarcoma, immunohistochemistry for pancytokeratin and vimentin was performed. Four µm sections were labeled by the

peroxidase method. Cytokeratin (clone AE1/AE3, Dako) and vimentin antibodies (clone VIM 3B4, Dako) were diluted 1:100 and applied to sections for 1 hour at 37°C. DAB served as the chromogen and Mayer's hematoxylin as the counterstain. Neoplastic cells were pancytokeratin negative (Fig. 5), with marked labeling of internal control epithelial cells such as faveolar epithelial cells. The immunohistochemistry protocol for detecting vimentin used in this case did not label cells that should be considered internal control cells (i.e. mesenchymal cells), and therefore results of vimentin staining were not considered. Together, the morphological features of the neoplasia and lack of cytokeratin expression supported the diagnosis of undifferentiated sarcoma.

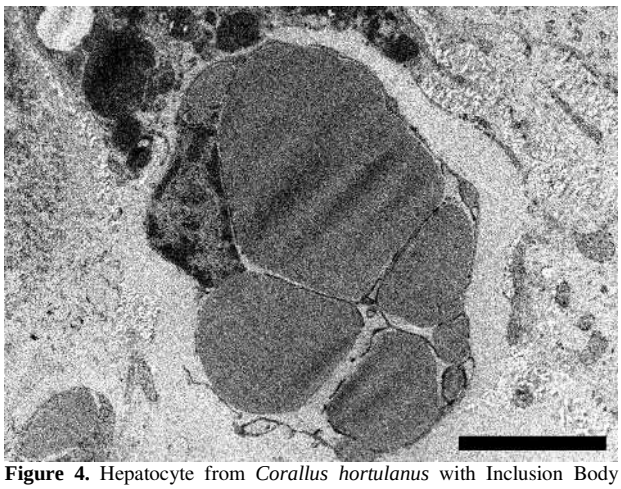


Figure 4. Hepatocyte from *Corallus hortulanus* with Inclusion Body Disease. Multiple intracytoplasmic electron-dense inclusion bodies. Uranyl acetate and lead citrate. Bar = 5 µm.

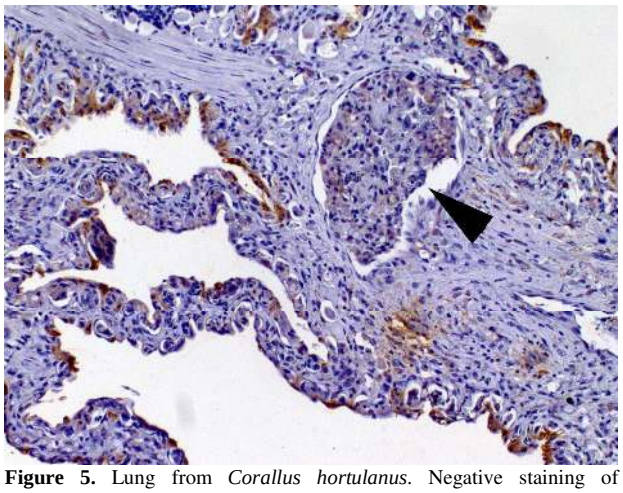


Figure 5. Lung from *Corallus hortulanus*. Negative staining of neoplastic sarcomatous cells for cytokeratin (CK AE1/AE3) in a pulmonary vascular embolus (arrow head). Faveolar epithelial cells are diffusely labeled. Immunohistochemistry stain with Mayer´s hematoxylin counterstain. 20x objective,

Discussion

To the best of our knowledge this is the first report of IBD in a *Corallus hortulanus*, and this is also the first report of IBD in Brazil. Intermittent regurgitation is often the first clinical sign of the disease, followed by anorexia that was observed in this case and lead to cachexia. Although neurological signs including behavioral changes, head tremors, ataxia, disorientation, opisthotonos, and flaccid paralysis have been described in cases of IBD, unfortunately, samples of the central nervous system were not available for histopathology in this case. Affected snakes often develop ulcerative stomatitis, pneumonia, dermatitis, and lymphoproliferative disorders, including cutaneous sarcoma and leukemia (1, 6, 7, 9, 11). Thus, the undifferentiated metastatic sarcoma associated with the IBD in this case is not an unusual finding in IBD. Clinical signs may be acute or last for several months. Snakes may also have a subclinical infection, with the formation of inclusion bodies, but absence of clinical signs, remaining as asymptomatic carriers in these cases (2, 9). Although blood samples were not available in the present case, blood exams of acutely affected boa constrictors diagnosed with IBD may present leukocytosis, relative lymphocytosis, lower total protein and globulin values and significantly higher aspartate transaminase values when compared to those of chronically affected snakes (9).

Ante mortem diagnosis of IBD is based on biopsies, especially of esophageal tonsils and liver. These tissues often have eosinophilic intracytoplasmic inclusions. Blood smears from suspect cases can be examined for the presence of inclusions in lymphocytes and heterophils, but the sensitivity is low (2, 9). A 68 kDa protein was identified as the protein accumulating in the inclusions and a monoclonal antibody has been developed against it and is being tested for its use in immunodiagnostic assays (12).

Post mortem diagnosis, as in this case, is based on gross findings and histopathology. Gross lesions include cachexia, pancreatic fibrosis, atrophy and fibrosis of the spleen, nodular lesions in the stomach and the esophagus, hepatic degeneration, ulcerative stomatitis, and pneumonia (2, 5, 9, 11). Detection of eosinophilic intracytoplasmic inclusions by histopathology is considered the definitive diagnosis (2, 9). Inclusions are usually found in visceral epithelia, especially in the liver (11). According to a previous report, inclusions are round to oval, 1-5 µm and methylene blue positive (7). In this case, the inclusions were toluidine blue positive and some were larger than previously described, may be due to a greater number of inclusions found. Ultrastructurally, inclusion bodies are visible as intracytoplasmic aggregates of granular electron-dense material that usually are not membrane limited. The transmission electron microscopy findings in this case are compatible with previous reports (7, 11, 12).

Until very recently, there was only a suspicion that IBD had a viral etiology. Many retroviruses were

investigated, but it is still unclear whether these viruses play a role in the pathogenesis of the disease (3, 4, 12). Using a metagenomic approach to search for candidate etiologic agents in snakes with confirmed IBD, two viruses related to arenaviruses were identified, implicating these viruses as candidate etiologic agents of the disease (10). Although there is evidence that IBD is contagious, the mode of transmission is unknown. The bloodsucking snake mite *Ophionyssus natricis* is a common problem in snake collections affected by the disease and it is thought to act as a vector, although currently there are no experimental evidences supporting this hypothesis (8).

The most important infectious differential diagnosis is paramyxovirosis, an infection that causes neurological and respiratory signs (11). However, while in IBD usually there is no tissue or cellular response associated to the numerous inclusions, in paramyxovirosis snakes often present hemorrhagic to necrotizing pneumonia associated to occasional eosinophilic intranuclear or cytoplasmatic inclusion bodies. Based on the histopathological findings, paramyxovirus infection was excluded in this case.

The general condition of affected snakes can sometimes be improved by force-feeding and rehydration. However, there is no current treatment for the disease, and, therefore, its control should be based on prevention (11). As there is no vaccine available, quarantine and liver biopsy are recommended before adding a new member to a collection (2).

Although in this case no neurological signs were noticed, the disseminated characteristic inclusion bodies are conclusive of IBD, which is a serious threat to captive collections of Boidae and should be included in the differential diagnosis of snakes presenting gastrointestinal, respiratory and neurological signs. In the present case, this was the first animal of Zoo-Botanical Foundation diagnosed with IBD. Therefore, further epidemiological analysis is needed.

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