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Case report

Pathological and Immunohistochemical Diagnosis of an Intestinal Leiomyosarcoma in a Zebra Finch

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Abstract

An adult male Zebra finch (*Taeniopygia guttata*) with blood in the feces was examined for an internal mass in the abdominal caudal region near to the cloaca. During the necropsy, a large tumor mass around the large intestine was observed. The histopathology was suggestive of smooth muscle tumor and the immunohistochemistry analysis was performed for vimentin, desmin, SMA and KIT for the identification of the tumor histogenesis. The results were consistent with intestinal leiomyosarcoma.

Key words: avian neoplasm, immunohistochemistry, histopathology, avian pathology, leiomyosarcoma.

Introduction

Primary neoplasms of the intestine include several types of sarcomas, present as masses within the intestine (17). Smooth muscle neoplasms termed leiomyomas (benign) and leiomyossarcomas (malignat) may arise from the intestinal muscular layers (5).

Intestinal primary neoplasm, although rare, have been reported in captive and free-ranging birds. Smooth muscle neoplasm frequently arises from splenic smooth muscle trabeculae, gastrointestinal tract, female reproductive tract, vas deferens and testicular capsule. Leiomyomas are nodular neoplasms that might be associated with abdominal distention, gastrointestinal or reproductive tract obstruction, or organ displacement, while leiomyosarcomas may be locally invasive and promote metastasis, although it is a late and infrequent event (10).

The diagnosis of tumors depends on their location, macroscopic and microscopic features. However, the determination of histogenesis and definitive diagnosis might need immunohistochemical staining with specific antibodies to the neoplastic cells in some cases (7).

Case Report

An adult male Zebra finch (*Taeniopygia guttata*) from a private owner arrived dead in a veterinary clinic with a history of bloody feces and depression. During the physical examination at necropsy, the bird had a large abdominal increase in the caudal region, near to the cloaca.

The solid mass sized about 4 cm, was well demarked, whitish, firm, and had rich vascularity on its surface. In the cut surface, it was observed a lobular formation with lamellar growth, adherent and infiltrating in the intestine. At necropsy, nodules compatible with metastases in other organs were not observed.

The tissue sample was collected and fixed in 10% neutral formalin for five days, routinely processed and embedded in paraffin, cut into sections of 5 µm thick, and stained with hematoxylin and eosin for histopathology or utilized for immunohistochemistry (IHC). For IHC, the streptavidine-biotine method was used with anti-smooth muscle alpha actin (SMA) (Dako m0851; 1:750), anti-vimentin (Dako m0725; 1:2500), anti-KIT (Cellmark 117r-16; 1:150) and anti-desmin (Dako m0760; 1:300), as primary antibodies. Human tissues were used as positive controls for immunohistochemistry, samples of tonsils

were used as control for SMA and vimentin, GIST for KIT and myomas for desmin. Briefly, after deparaffinization and rehydration of the tissue sections, the endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 min. The antigen unmasking was performed with a citrate buffer, pH 6.0 for 40 min at 95ºC in a microwave stove. Nonspecific endogenous protein was blocked with diluted normal serum and followed by the incubation with the primary antibodies. Sections were then sequentially incubated with the biotinylated secondary antibody and with the enzyme complex, followed by incubation (with the appropriated substrate-chromogen), counterstaining (with hematoxyilin), and mounting.

The diagnosis was based on the histological classification of mesenchymal tumors established by the World Health Organization (WHO) (8) and on the immunohistochemical classification by the Brazilian Society of Pathology (SBP) (19). Malignant tumors are graded I to III based on cellular differentiation, presence of necrosis within the neoplasm, and mitotic rate. The final score is designated in grades, being grade I (score 3-4), grade II (score 5-6), grade III (score >7) (2, 13).

On histopathology examination it was observed mesenchymal cells arranged in interlacing bundles with multifocal areas of coagulative necrosis. The cells exhibit a spindle cell shape with a vesicular cigar shaped nuclei. The mitotic activity was evaluated by the observation of the number of mitosis in 10 high power fields (HPF) with an average of 4.27 mitotic figures/10 HPF.

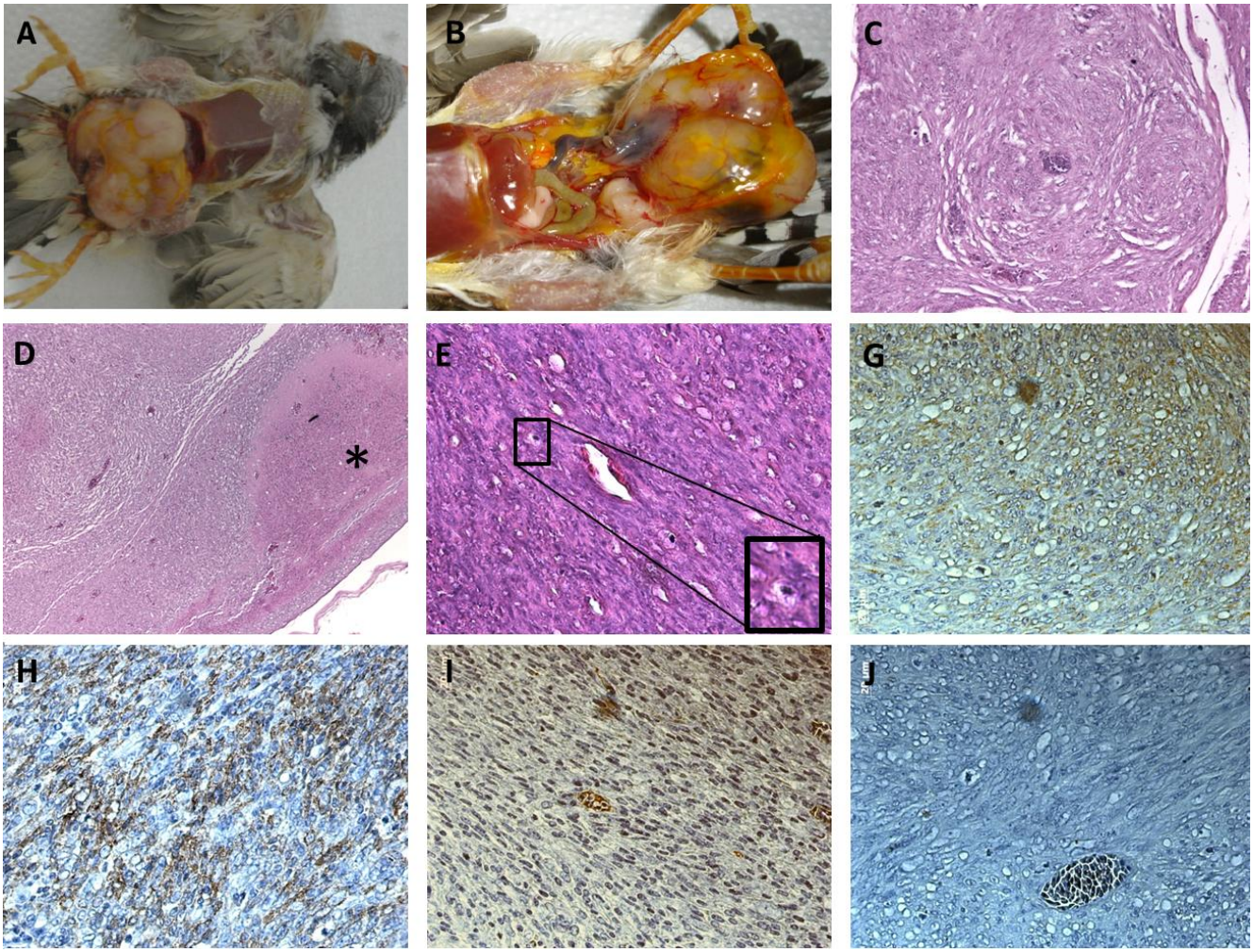

Figure 1. Leiomyosarcoma in Zebra Finch. Large whitish and firm mass involving whole intestine (A, B). Photomicrography of sections of the tumor stained with HE, showing interlacing fascicles and bundles (**C –** x200), with an area of focal necrosis (*****) (**D** – x50), the cell morphology shows elongated shaped spindle cells, intracytoplasmic vacuoles and cigar shaped nuclei, and presence of mitosis in detail (**F –** x400).Photomicrography of the immunohistochemistry sections of the tumor showing positive staining for desmin (**G** – x400), SMA (**H** – x400) and vimentin (**I** – x400); and negative staining for KIT (**J** – x400).

The immunohistochemistry assay was performed and showed weak positive cytosolic stain for the vimentin antibodies, strong positivity stain for SMA (1a4), desmin antibodies; and however, with the antibodies KIT (CD117) the neoplastic cells were negative, as shown in Figure 1. The histological and immunohistochemical results allowed the diagnosis of a well differentiated intestinal leiomyosarcoma (grade II) in this Zebra finch.

Discussion

The gross, histopathological and immunohistochemical findings were consistent with well differentiated leyomiosarcoma grade II in an adult male Zebra finch (*Taeniopygia guttata*).

Smooth muscle neoplasms, like leiomyosarcomas are the most common muscle neoplasm reported in captive and free-ranging birds (10). A review of articles that observed just by histology the incidence of neoplasms in avian species found 16 leiomyosarcomas in a study of 168 tumors in budgerigars (3); 100 cases of leiomyomas in 2281 cases of natural neoplasia in domestic fowls (15); five smooth muscle tumors in 383 naturally occurring neoplasms in birds (14), and, in a study of non-leukotic avian neoplasms, 17 cases out of 126 were leiomyomas (20).

Smooth muscle tumors present as masses within the intestinal wall. Histologically, they are comprised of interlacing bundles of cells with fibrillar cytoplasm and vesicular nuclei. Differentiation between leiomyoma and leiomyosarcoma depends on mitotic activity and the degree of cellular anaplasia (18).

Leiomyomas are defined but not encapsulated expansive tumors, composed of interlacing cells that resemble smooth muscle cells; in cross-section, the nuclei is centrally placed, while longitudinally, the nuclei appear "cigar-shaped". The nuclei lack anisokaryosis, but are scattered vesicular with prominent nucleoli. Mitotic figures are absent or rarely seen. Leiomyomas are immunohistochemically positive for desmin and smooth muscle actin, and sometimes positive for vimentin (2).

Grossly leiomyosarcomas resemble leiomyomas. Microscopically they are highly cellular, infiltrative tumours composed of arranged spindle cells with vesicular nuclei and prominent nucleoli. Anisokaryosis may be present. Mitoses are normally found with the average of four mitoses per ten high power fields (x400). Leiomyosarcomas are positive for smooth muscle actin, desmin and vimentin (2).

In human pathology, and more recently in veterinary pathology, many gastrointestinal mesenchymal neoplasms have been reclassified. Previously diagnosed smooth muscle tumors (leiomyosarcomas and leiomyomas) were found to exhibit immunoreactivity, genetic and ultrastructural properties more consistent with GISTs (gastro-intestinal stromal tumors) (6). Although these authors did not find in the literature the presence of GISTs in avian species, it is known that birds have interstitial cells of Cajal positive for KIT (21). Likewise, we believe it is interesting to use and search for cell markers to classify tumors and early identify avian patients and so evolve the avian pathology and medicine.

Regarding to the immunohistochemistry assay, antibodies for SMA, desmin, KIT and vimentin were tested to determine the histogenesis of the neoplasm. KIT, also known as c-KIT and CD 117, is a marker for Cajal cells, a spindle-shaped cell found in the myoenteric plexus between smooth muscle layers of the gastrointestinal tract of mammals and chickens (21). CD 117 is commonly used as a marker for GISTs in mammals (6). Vimentin is the major constituent of the intermediate filament family of proteins expressed in normal mesenchyma. Its overexpression is related to poor prognosis (16). Vimentin is always positive in case of GIST (2,6), however it is not always detected in cases of leyomiosarcoma (19). Desmin is a subunit of intermediate filament expressed in muscle tissues of vertebrates (12). SMA, or 1a4, is a cytoskeletal protein responsible for the anchorage of the myofibrilar actin filaments (16).

The results showed intracytoplasmatic positivity for vimentin, desmin and SMA and no immunoreactivity for KIT. The immunohistochemical results were consistent with the diagnosis of leiomyosarcoma, as previously reported in dogs (4, 9), pygmy sperm whale (11), and humans (16). The lack of immunoreactivity for KIT (CD117) excluded the diagnosis of GIST diagnosis and the lack of vimentin reactivity associated with smooth muscle actin SMA positive labeling was consistent with the immunoreactivity of leiomyocytes (11).

Although the authors did not found in the literature cases of leiomyosarcoma in Zebra finch and this is the first one with histological and immunohistological identification, we know that cases of smooth muscle tumors are common in birds. The human and veterinary medicine is evolving and increasingly using biomarkers to identify the origin and behavior of tumors. Birds are increasingly being used as pets and it leads to the increase in their quality of life and life expectancy. Consequently, it shall be found more cases of neoplasia in captive birds and, thus, it is imperative to use markers to differentiate and identify these tumors and be able to use specific methods of prevention and improve treatment.

In conclusion, although the authors know that cases of smooth muscle tumors are common in birds, they did not find reported cases of leiomyosarcoma in Zebra finches and this seems to be the first report including immunohistochemical diagnosis of intestinal leiomyosarcoma in this bird species. The human and veterinary medicines are evolving and increasingly using biomarkers to identify the origin and behavior of tumors. This makes important to report cases where the immunodiagnosis was successfully used in birds, as they are frequently utilized as pets, requiring more knowledge in the avian pathology field.

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