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Case Report

Proventricular and ventricular gastrointestinal stromal tumor (GIST) in a scarlet macaw (*Ara chloropterus*)

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Abstract
GISTs can be benign or malignant, and they have been reported in a wide variety of species, including humans, non-human primates, dogs, and cats; however, reports of this tumor in birds are rare. These neoplasms are seen in the gastrointestinal tract and are derived from the Cajal Cells responsible for regulating gastrointestinal motility. We report a case of a gastrointestinal stromal tumor in a scarlet macaw (*Ara chloropterus)* that was referred to necropsy at the Veterinary Hospital of the Federal University of Paraíba (UFPB), Northeastern Brazil. Macroscopically, the proventriculus and ventriculus were dilated, and the walls of both organs were thickened by a firm, ulcerated mass covered by fibrin strands. In the histopathology, the proventriculus presented an expansive, non-encapsulated neoplasm formed by homogeneous spindle cells, with eosinophilic cytoplasm, indistinct borders, mild to moderate pleomorphism, and rare mitotic figures, being suggestive of a leiomyoma. In immunohistochemistry, a positive multifocal immunolabeling for the C-KIT proto-oncogene was observed in the cytoplasm of neoplastic spindle cells, thus compatible with a gastrointestinal stromal tumor (GIST). The occurrence of GISTs in avian species might have been overlooked, and the authors recommend that previously reported gastrointestinal leiomyomas or leiomyosarcomas be tested for this immunomarker to rule out the possibility of GISTs.

Keywords: neoplasm, gastric tumors, Cajal cells, parrots.

Introduction

Gastrointestinal tumors form a heterogeneous group of neoplasms with distinct morphological, histopathological, and molecular features (35). Mesenchymal and epithelial neoplasms are the most common gastrointestinal tumors, and they originate mainly from the smooth muscle and glandular epithelium, respectively (1, 35). In birds, these tumors may be located at a wide variety of sites, ranging from the oral cavity to the final portion of the intestine, with adenocarcinomas, squamous cell carcinomas, and leiomyomas being the most reported neoplasms. (2, 9, 26).

Among the mesenchymal neoplasms of the gastrointestinal tract in humans, gastrointestinal stromal tumors (GISTs) are the most reported, followed by smooth muscle neoplasms, such as leiomyomas and leiomyosarcomas (1). GISTs are neoplasms seen in the gastrointestinal tract and derived from the Cajal Cells, responsible for regulating gastrointestinal motility (14). Microscopically, GISTs are characterized by a homogeneous, non-encapsulated, expansive mass associated with mild to moderate pleomorphism and mitotic activity, especially in regions of high cellularity (20, 22).

GISTs can be benign or malignant, and they have been reported in a wide variety of species, including humans, non-human primates, dogs, and cats (10, 29). However, reports

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of GIST in birds are rare. Here, we report a case of a gastrointestinal stromal tumor in a scarlet macaw (*Ara chloropterus)* that was referred to necropsy at the Veterinary Hospital of the Federal University of Paraíba (UFPB), Northeastern Brazil.

Case Description

An adult female scarlet macaw (*Ara chloropterus*) that weighed 0.805 kg was referred to necropsy at the Laboratory of Veterinary Pathology at the Federal University of Paraiba (HUV-UFPB), Areia-PB, with a clinical history of sudden death from a suspected capture myopathy.

External examination revealed that the animal was cachectic and apterya in the pectoral region was noted (Fig.1A). After opening of the celomatic cavity, the proventriculus and ventriculus were dilated (Fig.1B). The wall of both organs were thickened by a firm, ulcerated mass covered by fibrin strands (Fig.1D). The ventriculus contained a blackened content (blood) adhered to the koilin membrane (Fig.1C). The liver appeared diffusely pale, increased in size, and with a thickened Glisson's capsule. Within the intestinal lumen, the presence of nematodes was observed. Cytology of the mass by fine-needle aspiration was then performed, and individualized spindle cells with moderate pleomorphism were observed.

All organs were collected, fixed in 10% formalin, trimmed, and sent for routine histopathological processing. The slides were cut at 5µm and stained with Hematoxylin and Eosin (HE). Microscopically, the proventriculus presented an expansive, non-encapsulated neoplasm formed by homogeneous spindle cells with eosinophilic cytoplasm, indistinct borders, mild to moderate pleomorphism and rare mitotic figures (Fig. 2A). These cells were arranged in large bundles and interlacing fascicles, indicating a mesenchymal neoplasm suggestive of a leiomyoma (Fig. 2B). The intestine exhibited moderate necrosis with the detachment of enterocytes. Within the intestinal lumen, large parasitic structures were sectioned both in cross-section and longitudinally, revealing a thick cuticle, a celomic cavity, and a digestive and reproductive tract that contained eggs compatible with nematodes.

Subsequently, the histological sections of the proventricular and ventricular neoplasia were submitted to immunohistochemistry. Briefly, histological sections of 4 µm

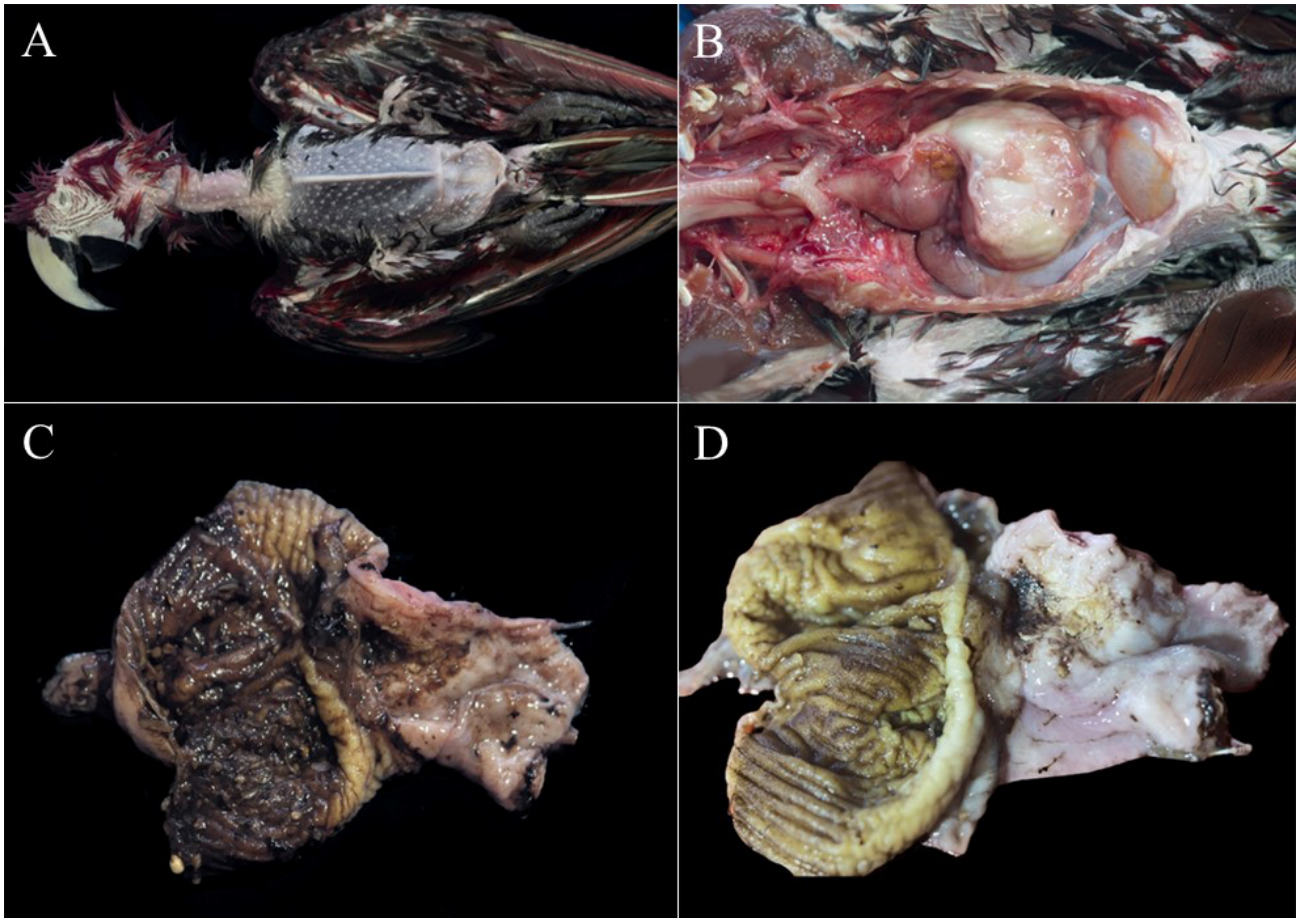

Figure 1. Anatomopathological findings of a proventricular gastrointestinal stromal tumor in a scarlet macaw (*Ara chloropterus*). A- On external examination, there is a focally extensive area of apteria in the pectoral region. B- The proventriculus is dilated and has a thickened wall. C- There was a blackened content adhered to the coilin membrane, compatible with blood. D- The mass was firm, ulcerated, and contained fibrin filaments, compressing the proventricular wall.

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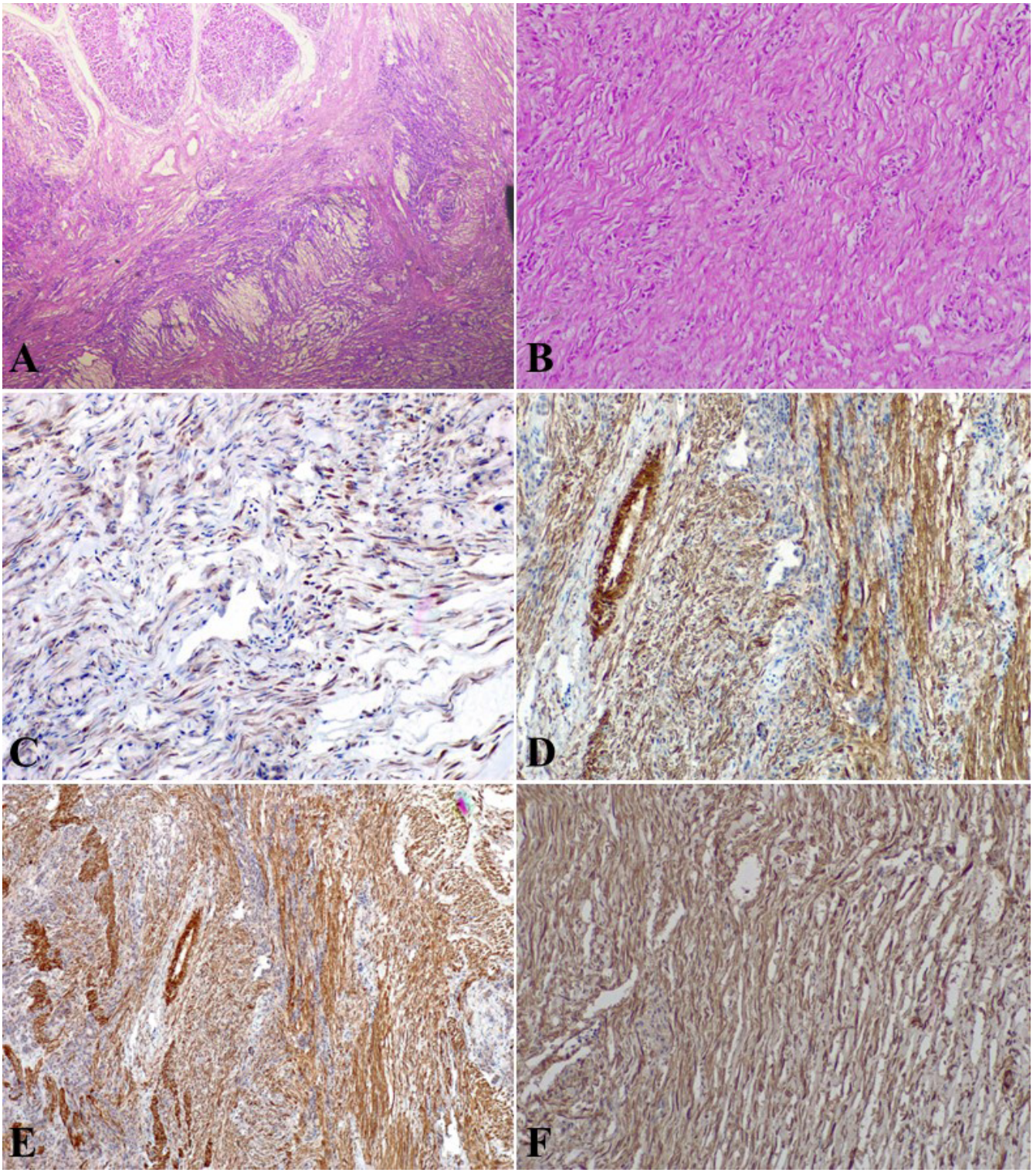

Figure 2. Histopathological findings and immunohistochemistry of proventricular gastrointestinal stromal tumor in a scarlet macaw. A- Loss of architecture and replacement of proventriculus glands due to infiltration of spindle neoplastic cells arranged in large bundles. Obj. 20x. HE. B- Presence of a non-encapsulated mass formed by a population of fusiform cells with eosinophilic cytoplasm, with poorly delimited borders and intertwined fascicles. Obj. 2,5x. HE. C- Muscle spindle cells of neoplasm demonstrating immunoreactivity for C-KIT proto-oncogene (CD-117). Obj. 10x. D- Positive immunolabeling in muscle mesenchymal cells for an immunomarker of smooth and skeletal muscle differentiation (muscle-specific actin) Obj. 20x. E- Another positive field of mesenchymal cells for specific muscle actin (HHF-35). Obj. 10x. F- Muscle spindle cells were immunolabeling for Alpha Smooth Muscle Actin. Obj. 20x.

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were deparaffinized in xylene, hydrated in decreasing concentrations of ethanol, and washed in distilled water, being submitted to antigenic recovery by heat in high pH solution (Target Retrieval solution High pH-DM828, K800221-2 EnV FLEX+, High pH Link, DAKO) in a pressure cooker (PascalR, Dako). The slides were placed at room temperature for 20 minutes for cooling and washed with deionized water and endogenous peroxidase blockade by immersion in ready-to-use hydrogen peroxide. Then, the sections were washed in tris solution (pH 7.4), and the nonspecific sites were blocked with protein block serum-free solution (DAKO, X0909). Theprimary antibody (polyclonal CD117) was incubated for 18 hours at 4°C. The amplification and detection system used was EnVision FLEX/HRP, SM802 (Dako) and the chromogen diaminobenzidine (EnVision FLEX DAB+CHROMOGEN, DM827, DAKO and EnVisionTM FLEX PEROXIDASE-BLOCKING REAGENT SM801, K800221-2 EnV FLEX+, High pH Link, DAKO). The following positive external controls were used: mast cell tumor for C-KIT, vasculature for smooth muscle actins (HHF-35 and 1A4), and striated muscle for Desmin (D33). Slides were counterstained with Harris haematoxylin. Antibodies against alpha-smooth muscle actin (1A4), polyclonal CD-117 (C-KIT), muscle-specific actin (HHF-35), and Desmin (D33) were used.

A positive multifocal immunolabeling for C-KIT proto-oncogene was observed in the cytoplasm of neoplastic spindle cells (Fig. 2C). These mesenchymal cells arranged in fascicles also demonstrated diffuse cytoplasmic positive immunolabeling for alpha (Fig. 2D-E) and specific (Fig. 2F) smooth muscle actin. Cells were negative for Desmin (D33); only the internal control showed positivity. The macroscopic, histopathological, and immunohistochemical findings were compatible with a gastrointestinal stromal tumor.

Discussion

In the present report, the low body score of the bird, associated with a firm and ulcerated mesenchymal neoplasm that expanded the proventriculus and presented positive immunolabeling for C-KIT and negative expression for Desmin was indicative of a gastrointestinal stromal tumor (GIST).

Despite the distribution of GIST in any portion of the gastrointestinal tract, in mammals, this neoplasm is mainly found in the stomach, with a frequency of 55.6% of cases in humans (22), which corroborates with the location of the tumor in the case reported here. Gastrointestinal stromal tumors (GISTs) exhibit a high mutation rate in the KIT gene that can influence tumor progression through slow or rapid growth, and this mutation is a possible factor in the development of the neoplasm in this case. (3, 11).

Depending on the growth rate of the GISTs, the masses can rupture and form areas of ulcerations that cause gastric hemorrhages (21, 38, 39), as seen in the ventricular lumen of the present case. These tumors may also be related to the development of bleeding due to mucosal compressions and gastric dilations secondary to the infiltration of neoplasms, thus leading to progressive weight loss and a low body score (21, 27), as observed in the macaw.

Descriptions of gastrointestinal stromal tumors in birds are scarce. Only one case in a red-tailed hawk (*Buteo jamaicensis*) was reported as an uncommon mesenchymal tumor in the proventriculus, where the authors considered GIST as one of the differential diagnoses. However, the immunohistochemical panel used for this neoplasm did not show specific positive immunolabeling for C-KIT or vimentin, making the diagnosis of GIST inconclusive (33). In reports of brown rats (*Rattus novergicus*), chimpanzees (*Pan troglodite*s), ferrets (*Mustela putorius furo*), and Spanish ibex (*Capra pyrenaica hispanica*), the histological patterns of spindle cells arranged in interlaced bundles led to previous diagnoses of leiomyomas or leiomyosarcomas that resemble the features described in GISTS. However, the positive immunolabeling for C-KIT (7, 12, 29, 34), as in this scarlet macaw, differentiated and defined these cases as gastrointestinal stromal tumors. The difficulty distinguishing smooth muscle neoplasms from gastrointestinal stromal tumors may explain the rare reports of these tumors in birds.

Macrorhabdiosis, proventricular dilatation disease (PDD), and lead poisoning also lead to proventricular dilation and thickening in psittacine birds and must be considered as differential diagnoses (16, 25, 26). One of the main differentials of gastrointestinal stromal tumors is smooth muscle neoplasms, such as leiomyomas and leiomyosarcomas. These neoplasms are composed of spindle cells arranged in interlaced bundles. They are non-encapsulated and have intense and diffuse cytoplasmic immunoreactivity to vimentin and smooth muscle alpha-actin. However, they do not express immunolabeling for C-KIT. (4, 19, 40). Neoplasms of epithelial origin, such as proventricular adenocarcinoma, are considered possible differentials in this case due to mucosal thickening and proventricular dilatation in addition to strong cytoplasmic immunolabeling of neoplastic cells for pancytokeratin (2, 26, 37).

Histological features seen in the present case, such as the presence of homogeneous spindle cells arranged in interlaced bundles with eosinophilic cytoplasm and elongated nuclei, are common features described in other cases of the same neoplasm in baboons (*Papio anubis*), chimpanzees (*Pan troglodytes*), spider monkeys (*Ateles paniscus*), and domestic dogs (*Canis lupus familiaris*) (3, 10). The histopathological patterns of these neoplasms can be classified into three types: mesenchymal, epithelioid, and mixed. Seventy percent of the presentations involve mesenchymal patterns that are characterized by eosinophilic cells and slightly pale cytoplasm arranged in fascicles, being initially similar to leiomyoma (6) as observed in the tumor reported here.

In mammals, GISTs show positive cytoplasmic immunolabeling for C-KIT and Smooth Muscle Actin (10). In domestic chickens, Cajal interstitial cells were found mainly in the muscle and submucosal layers, demonstrating strong

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immunolabeling for C-KIT (36), as well as in the proventriculus and ventriculus of this scarlet macaw. The positive immunolabeling for CD-117 (C-KIT) is attributed to a mutation in the KIT gene of gastrointestinal stromal tumors, which is present in approximately 90% of these neoplasms (11). In this study, additional markers demonstrating positive expression include alpha-smooth muscle actin and specific smooth muscle actin. These markers display reactivity in approximately 25% of cases among domestic animals and humans (10, 23). Desmin has negative expression in most cases of GIST (20), thus confirming the findings in relation to this proventricular and ventricular neoplasm.

GISTs should be considered as differential diagnoses for proventricular or ventricular dilatation in birds, as well as for mesenchymal gastrointestinal neoplasms in avian species. Definitive diagnosis of GIST requires positive immunolabeling for C-KIT. The occurrence of GISTs in avian species might have been overlooked, and the authors recommend that previously reported gastrointestinal leiomyomas or leiomyosarcomas be tested for this immunomarker to rule out the possibility of GISTs. To the best of the authors' knowledge, this is the first report of a confirmed GIST in an avian species.

Conflict of Interest

The authors declare no competing interests.

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