

### Case Study

## A rare case of cutaneous leiomyosarcoma in budgerigar (*Melopsittacus undulatus*)

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### ABSTRACT

Leiomyosarcoma in birds is relatively rare. This tumor as a muscle neoplasm was reported in captive and free ranging birds. Smooth muscle cells may develop to the leiomyosarcoma, but splenic smooth muscle trabeculae is most common site of the tumor growth. Although budgerigars have an incidence of neoplastic diseases but smooth muscle tumors were rarely reported in this species. To the best of our knowledge, present case is the first report of cutaneous leiomyosarcoma in budgerigar. The bird was referred with a history of growing mass in subcutaneous tissue of abdomen. First bird was suspected to the egg impaction, but necropsy confirmed a firm, creamy structure that suspected to the tumor mass. No invasion was observed to the other organs. After excision of the mass, routine histopathologic evaluation and immunohistochemistry investigation were performed for desmin and smooth muscle actin (SMA). Histopathologic examination revealed spindle-shaped cell with cigar-shaped, round, or oval nuclei with cytologic criteria of malignancy including marked pleomorphism and high mitotic activity. These findings were consistent with immunohistochemistry profile of our case and thus confirmed as cutaneous leiomyosarcoma.

**Keywords:** Budgerigar, cutaneous, leiomyosarcoma

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### INTRODUCTION

Smooth muscle neoplasms occur through some possible mechanisms including pluripotent cells of the embryonic structure, neoplastic transformation of myoblast cells or embryonic remnants of myotomes. Frequency of the smooth muscle tumor was estimated

about two fold striated muscle tumor incidence. In addition, muscle malignant tumors are more frequent than benign ones (Moulton 1990, Latimer 1997). Leiomyosarcoma in birds was occurred in different site including crop, intestinal tract, pancreas, oviduct, trachea, and ventral ligament of the oviduct, but splenic trabeculae is the most common site of the tumor growth (Latimer 1997). Cutaneous leiomyosarcoma as a soft tissue sarcoma is extremely rare in human being

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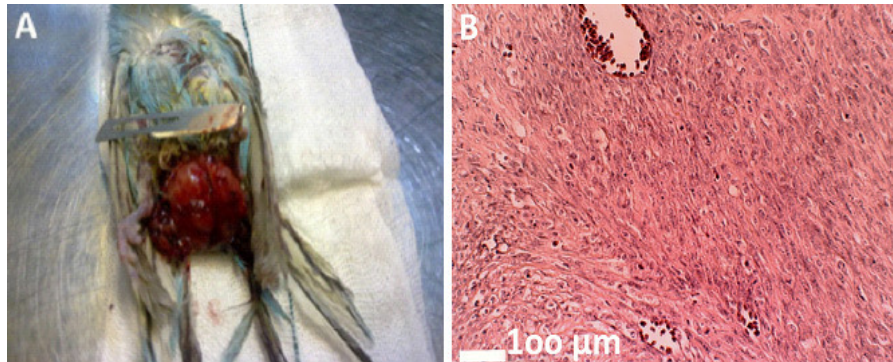
(Humphreys *et al.* 2004). In addition, leiomyosarcoma of the skin and subcutaneous tissue is a very rare tumor in domestic animal (Movassaghi and Sardari 2008). A few reported cases in the literature are dermal intravascular leiomyosarcoma in an adult cat (Jacobsen and Valentine 2000) and subcutaneous leiomyosarcoma in a cow (Brunnert *et al.* 1990). However similar to the domestic animals, smooth muscle tumors in birds are rare with reported incidence of 0%, 1.9%, 4.9%, and 7% in different studies (Beach 1962). In birds, cutaneous leiomyosarcoma has been reported in a pigeon (Movassaghi and Sardari 2008). To the best of our knowledge current case is the first report of cutaneous leiomyosarcoma in budgerigar.

#### CASE HISTORY

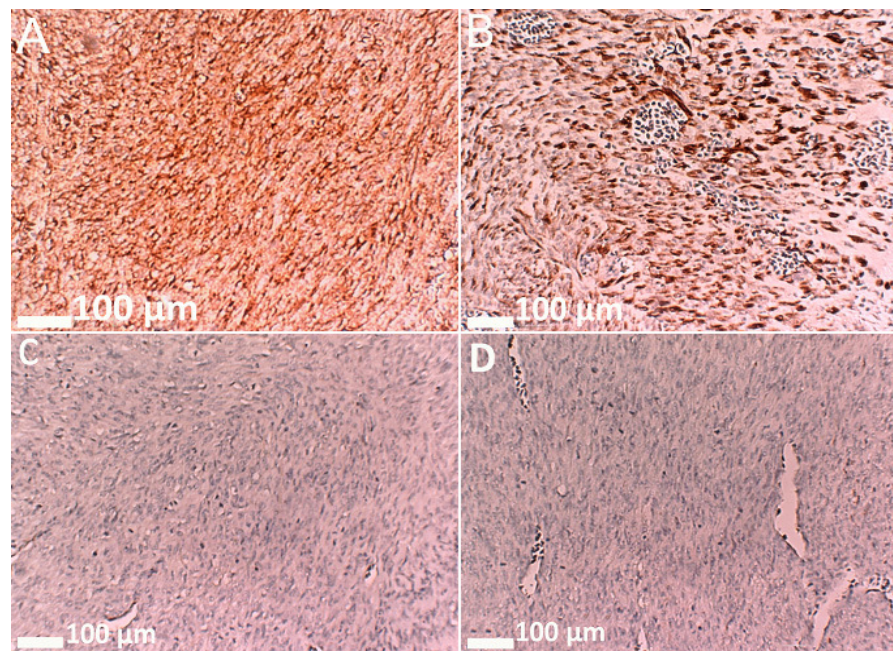
A 6-year-old, female budgerigar presented with a growing mass in the abdominal region was referred. On initial examination, the bird was suspected to have egg impaction. After a few days, the bird's general condition deteriorated and it died within 7 days; the animal was submitted for necropsy. At necropsy, a subcutaneous tumor mass (2 cm diameter) appearing creamy and solid was found. The tumor lacked a true capsule, and it was limited to the cutaneous and subcutaneous portion. No invasion was observed to the abdominal cavity. Tumor slices were processed for histopathological, as well as, immunohistochemical examination. Immunohistochemistry (IHC) staining for smooth muscle actin (SMA) and desmin was performed for a final diagnosis. For IHC, paraffine-embedded tissues were sectioned (5 micron), mounted on 3-aminopropyl-triethoxy-silane-coated slides (Dako, Produktionsvej 42, 2600 Glostrup, Denmark), and air-dried overnight at 37 °C. Prepared slides were deparaffinized in three washes of xylene for 5 minutes each, and rehydrated in graded ethanol. After rinsing with water, endogenous peroxidase in the sample was blocked using 3% hydrogen peroxide for 30 min at room temperature. Then, sections were rinsed gently with water, followed by PBS, and then autoclaved in 0.1 mol/L citrate buffer at pH 6.0 for 30 min. After

cooling at room temperature, slides were rinsed with PBS for 30 min, incubated with a relevant primary antibody against SMA and desmin (both from Dako, Produktionsvej 42, 2600 Glostrup, Denmark) for 30 min, and then washed 2 times with PBS. Thereafter, sections were colored with Dako EnVision + Dual Link System HRP kit according to the manufacturer's instructions (Dako, Produktionsvej 42, 2600 Glostrup, Denmark). 3, 3'-Diaminobenzidine (Dako, Produktionsvej 42, 2600 Glostrup, Denmark) was used as chromogen to produce a brown-colored signal. The sections were finally counter-stained with Mayer's hematoxylin.

After mass excision, tumor was firm, creamy and without capsule (Figure 1A). Other organs in abdominal and thoracic cavity were normal and no invasion was observed to them. On tumor sectioning, a firm body with minor bleeding was observed. On histopathologic examination the tumor mass was densely cellular. Spindle-shaped cell were arranged in bundles; occasionally groups of oval-round shaped, pleomorphic cells were observed. Malignancy criteria included marked pleomorphism, anisokaryosis, hyperchromatism, and a high number of mitotic cells (15 per high power field). Cellular cytoplasm was slightly eosinophilic and the nuclei were predominantly very pleomorphic from cigar-shaped to round oval. Nuclei were majorly central and chromatin was finely granular. No region of necrosis or hemorrhage was observed in histopathology evaluation. A tentative diagnosis of cutaneous leiomyosarcoma was made (Figure 1B). Immunohistochemical pattern of intermediate filaments including desmin and SMA was also evaluated for confirming the diagnosis. These cells showed strongly immunoreactivity to SMA and Desmin, in more than 90% of cells stained for both markers. SMA and desmin immunostaining was cytoplasmic and varied in intensity (Figure 2A and B) (Negative controls were provided in Figure 2C and D). Staining intensity was more prominent in actin than desmin section. Because desmin-positive cells were randomly distributed, they were clearly distinguishable from non-reactive cells.



**Figure 1.** A: tumor mass in caudal abdominal region of budgerigar carcass. B: histopathologic section of the tumor mass. densely packed bundles of spindle-shaped neoplastic cells are seen at low objective. Cells show malignant features such as marked cellular and nuclear pleomorphism, and high mitotic figures (H&E).



**Figure 2.** Immunohistochemical (IHC) staining of the diagnosed leiomyosarcoma using antibody against smooth muscle actin (SMA) and desmin (B) filaments. High expression of smooth muscle actin (SMA) (A) and desmin (B) markers by neoplastic cells are seen. Staining intensity was higher in SMA section than desmin section. Negative control for SMA (C) and desmin (D).

## DISCUSSION

Although neoplastic diseases were frequently reported in domestic and exotic birds, but neoplasm of the striated and smooth muscle is one of the rare neoplasms in birds. Smooth muscle tumors include leiomyoma and leiomyosarcoma. Leiomyomas commonly have nodular pattern and generally occurred in female reproductive and gastrointestinal tract. On the other hand, leiomyosarcoma as malignant tumor occurred commonly in splenic trabeculae. Malignant tumors of smooth muscle constitute only 10% of the total smooth muscle tumors reported (Hulland 1990).

Smooth muscle tumors containing leiomyosarcoma and leiomyoma may originate from arrector pili muscle, cutaneous blood vessels (angioleiomyoma) and muscles of genital skin. These tumors are solitary and firm in animals (Gin *et al* 2007, Hulland 1990) as we found in this budgerigar. Because both leiomyoma and leiomyosarcoma occur in similar sites, it suggested that leiomyosarcoma may result from malignant transformation of leiomyoma. Leiomyosarcoma is reported commonly in dogs (Hulland 1990). However, leiomyosarcoma in birds is relatively rare and most commonly reported sites are internal organs, especially

spleen or genitalia. Although, leiomyosarcoma may occur in different anatomic locations of the alimentary tract including crop, jejunum, and lower intestine (Beach 1962, Blackmore 1966, Petrak *et al* 1982, Potter *et al* 1983), in poultries, mesosalpinx is the most common site of this tumor (Movassaghi and Sardari 2008). Myxoid leiomyosarcoma of the gizzard and jejunal leiomyosarcoma also were reported in broiler chicken (Sato *et al* 2002). This report is description of cutaneous leiomyosarcoma in budgerigar. Histopathologic findings including cellular morphology and malignancy criteria showed origin and malignancy of the tumor as cutaneous leiomyosarcoma. It's necessary to differentiate leiomyosarcoma from fibroma or fibrosarcoma based on immunoreactivity for smooth muscle markers including desmin and SMA (Gin *et al* 2007). Strongly positive staining for desmin and SMA results confirmed the histopathologic diagnosis.

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