

## Case Report

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### Subcutaneous Haemangiosarcoma in a Cockatiel (*Nymphicus hollandicus*)

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#### Summary

An ulcerated, 1 × 0.5 cm, subcutaneous mass on the cranio-lateral aspect of the right tibiotarsus of a 4-year-old male cockatiel was removed. Histologically, the neoplasm was non-encapsulated, infiltrative and composed of irregular vascular channels lined by branching and variably sized spindle-shaped cells with large vesicular nuclei, prominent nucleoli and rare mitoses. Surrounding these vascular channels were fibroblasts and mixed inflammatory cells. Neoplastic cells had diffuse immunoreactivity to factor VIII supporting a diagnosis of haemangiosarcoma.

#### Case report

A 4-year-old male cockatiel presented with a 1 × 0.5 cm diameter red, irregular, ulcerated mass that protruded *c.* 2 mm from the skin surface of the cranio-lateral aspect of the right tibiotarsus. A biopsy of the mass was fixed in 10% buffered formalin and submitted to the University of Georgia Tifton Veterinary Diagnostic and Investigational Laboratory. The fixed tissue was routinely processed, paraffin embedded, sectioned at 4–5 µm thickness and stained with haematoxylin and eosin for light microscopic examination. Additionally, tissue sections were subjected to immunohistochemical (IHC) staining using the Universal DAKO LSAB2 system and 1 : 50 dilution rabbit polyclonal anti-human factor VIII-related antigen (Dako Corporation, Carpinteria, CA, USA). For this staining procedure, paraffin sections were cut at 4–5 µm, placed on glass slides, air dried, deparaffinized and rehydrated. For antigen retrieval, the slides were placed in Dako's target retrieval solution (S1700), steamed for 20 min, cooled and then rinsed with distilled water. Endogenous peroxidases were blocked using hydrogen peroxide and methanol for 15 min. The slides were then rinsed with distilled water. The factor VIII-related antigen antibody was diluted 1 : 50 and applied to the sections for 1 h. Next, the biotinylated linking antibody was applied to the sections for 20 min. The slides were then rinsed and enzyme-conjugated streptavidin was applied to the sections for 20 min, followed by a final rinse. The liquid DAB substrate (Dako) was applied to the sections for 7 min. The slides were then counterstained with haematoxylin, dehydrated and coverslipped. The normal blood vessel endothelium served as an internal positive control for the immunohistochemical stain. For a negative control, sections were processed as

described except that the primary antibody was withheld. For comparison to a species in which this immunohistochemical stain is routinely used for similar diagnostic evaluation, tissue from a canine with a similar mass was processed along with the cockatiel tissue.

Microscopic examination of the mass revealed a highly cellular, expansile and infiltrative, non-encapsulated neoplasm. The neoplasm was composed of irregular vascular channels that were lined by branching and variably sized spindle-shaped cells with single or occasionally binucleated vesicular nuclei and single to multiple prominent nucleoli. Mitotic figures were rare. Loss of nuclear polarity was observed in some areas of the tumour and nuclei seemed to be in close contact with each other. Surrounding these vascular channels were fibroblasts and mixed inflammatory cells. Occasional multinucleated cells were seen. There were multifocal areas of epidermal ulceration and fibrinocellular exudate. These areas contained mixed inflammatory infiltrates composed of heterophils, macrophages, lymphocytes and plasma cells. Factor VIII-immunostained sections showed strong cytoplasmic staining of the tumour cells (Fig. 1).

A diagnosis of haemangiosarcoma was made based on macroscopic and microscopic examination and immunohistochemical staining of the tumour. In this particular case, the designation of haemangiosarcoma rather than haemangioma was chosen despite a relatively low mitotic index due to the high number of atypical and pleomorphic cells and invasion to edges of the biopsy specimen. Cells were anaplastic, had large vesicular nuclei and often multiple large nucleoli. Scattered multinucleated cells often were observed. The tumour recurred locally within 2 months and the owner elected euthanasia. A necropsy was not performed and therefore it is not known if any metastasis was present.

#### Discussion

Haemangiosarcomas, also known as angiosarcomas, often are highly malignant tumours associated with a poor prognosis and have been reported in a wide range of species in veterinary medicine (Alian et al., 2000; Headley, 2005). Haemangiosarcomas commonly occur as visceral masses of the spleen, liver or other abdominal organ; however, haemangiosarcomas also are frequently found in non-visceral locations such as the skin and subcutaneous tissues (Alian et al., 2000; Headley, 2005).

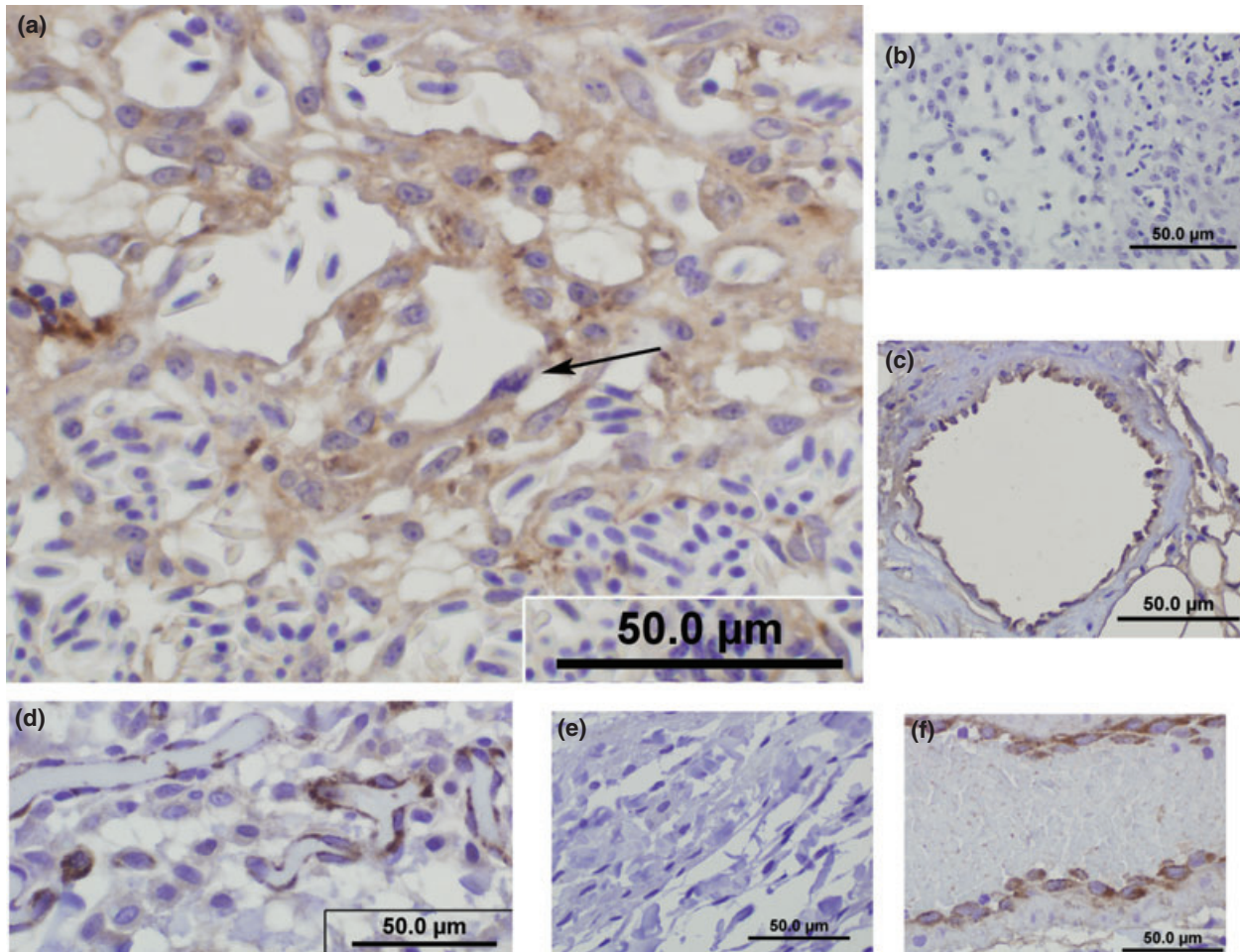


Fig. 1. (a) Section of a subcutaneous haemangiosarcoma in a 4-year-old male cockatiel showing irregular vascular channels, a rare mitotic cell (arrow) and positive immunohistochemical staining of tumour cells for factor VIII. (b) Negative control for the immunohistochemical staining of the cockatiel tumour. (c) Positive control demonstrating positive immunohistochemical staining for factor VIII in the endothelium of a normal blood vessel in the cockatiel. For comparison, (d), (e) and (f) show positive immunohistochemical staining of tumour cells (d), negative (tumour) control (e) and positive (endothelium of a normal blood vessel) control (f) in a canine.

Haemangiosarcomas are frequently seen in mammals and have mainly been reported in dogs and cats but have been seen rarely in other animal species (Tracy et al., 1985; Soffer et al., 1990; Robinson and Maxie, 1993; Rossi, 1998; Alian et al., 2000; Suedmeyer et al., 2001; Goldschmidt and Hendrick, 2002; Schultheiss, 2004; Headley, 2005). Histologically, these tumours have various morphologic appearances ranging from dense sheets of pleomorphic spindle cells with small rudimentary vascular structures interspersed throughout to cavernous or sieve-like masses with multiple larger vessels. In general, the prognosis for haemangiosarcoma is guarded. Metastasis of primary tumours to secondary locations (e.g. the lungs) is common especially with visceral tumours. Additionally, secondary complications such as gross haemorrhage or thrombosis often ensue (Alian et al., 2000; Schultheiss, 2004; Headley, 2005).

In avian species, tumours of endothelial origin, such as haemangiosarcomas and haemangiomas, have primarily been reported as flock problems in chickens (Tracy et al., 1985; Soffer et al., 1990; Goldschmidt and Hendrick, 2002). In poultry species, tumours of endothelial origin have been described and associated with a viral aetiology (Masegi et al.,

1993). Avian retrovirus has been linked to an increased propensity for developing haemangiomas in chicken especially in the spleen, muscle, skin and thymus (Tracy et al., 1985; Goldschmidt and Hendrick, 2002; Schultheiss, 2004). Few reports of tumours of endothelial cell origin have been documented in non-poultry avian species (Robinson and Maxie, 1993; Rossi, 1998; Suedmeyer et al., 2001). Cases of visceral haemangiosarcoma have been described in an ostrich and a parrot (Robinson and Maxie, 1993; Rossi, 1998). In both cases, complications from these tumours were suspected as reasons for death. A subcutaneous haemangiosarcoma has been described in a report from a golden pheasant (Suedmeyer et al., 2001). In this instance, as in our case, the tumour recurred soon after removal and the bird was killed. In the reports from the ostrich, parrot and pheasant, clinical evidence of a viral aetiology was not noted. This, however, does not preclude the possibility of a subclinical infection. Similarly, in our case, we cannot rule out the possibility of a subclinical viral infection. This is, to our knowledge, the first report of a subcutaneous haemangiosarcoma in a cockatiel and the first demonstration of positive factor VIII immunohistochemical staining to aid in said diagnosis in an avian species.

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