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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Neoplasia in Three Aye-Ayes (*Daubentonia madagascariensis*)

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Summary

Tumours diagnosed in three aged captive aye-ayes (*Daubentonia madagascariensis*), held in two different institutions, are described. A cerebral glioblastoma was diagnosed based on histological and immunohistochemical findings in one of the animals following initial presentation with bilateral mydriasis, absent pupillary reflex, head tilt and ataxia. A second animal was humanely destroyed due to impaired locomotion associated with spondylosis and a post-mortem diagnosis of cholangiocarcinoma was made based on histology with further confirmation with immunohistochemical labelling for cytokeratin 7. A third aye-aye suffering from dental disease was diagnosed with an oral squamous cell carcinoma following an excisional biopsy from a non-healing wound in the lip. Due to progression of the neoplasia the animal was humanely destroyed and post-mortem examination revealed the presence on an additional unilateral phaeochromocytoma.

Keywords: aye-aye; *Daubentonia madagascariensis*; immunohistochemistry; neoplasia

Neoplasia in aye-aye (*Daubentonia madagascariensis*) has not been reported despite previous reviews of neoplasia in prosimians (Remick *et al.*, 2009). A potential explanation for the absence of reports may be the relatively small captive population; the current captive population is only 57 animals, with only 59 deaths reported in aye-ayes over 1 year old in captivity according to the studbook for this species, currently classified at endangered by the International Union for Conservation of Nature (Andriaholinirina *et al.*, 2014).

Case 1, a wild-caught, intact, female aye-aye, estimated as 25 years old, presented with sudden onset of anorexia, obtunded mentation and truncal ataxia. Physical examination under anaesthesia revealed bilateral mydriasis and lack of pupillary light reflexes. Haematological and serum biochemical analyses and *Toxoplasma gondii* serology did not reveal any abnormalities and urinalysis results were within normal limits.

Because an episode of apnoea occurred during anaesthesia, the animal was allowed to recover from anaesthesia without performing any additional diagnostic tests. Despite showing increased activity on the following day, the clinical signs persisted and the animal was found dead 12 h later. The most relevant lesions during gross post-mortem examination were noted in the examination of the skull and brain. A 2–3 cm diameter red–white, soft, non-encapsulated but well demarcated mass was present in the rostroventral part of the right frontal lobe causing a 1.5 × 2.3 cm clearly-defined depression in the right olfactory bulb (Supplementary Fig. 1). In

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addition, there was marked asymmetry of the caudal portion of the nasal cavity, with destruction of nasal turbinates on the right side.

Tissue sections were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin (HE). Immunohistochemistry (IHC) for characterization of expression of glial fibrillary acidic protein (GFAP) (Dako/Agilent, Agilent Technologies, Stockport, Cheshire, UK; catalogue number Z0334; 1 in 2,000 dilution) and Olig2 (Linaris Biologische Produkte GmbH, Dossenheim, Germany; catalogue number PAK0107; 1 in 200 dilution) was carried out using a standard protocol employing an automated IHC system (Dako Autostainer; Dako/Agilent). IHC for Ki67 (Dako; MIB-1; 1 in 150 dilution), alpha thalassaemia with mental retardation linked to chromosome X (ATRX) (Atlas Antibodies, Cambridge Bioscience, Cambridge, UK; 1 in 1,000 dilution) and isocitrate dehydrogenase-1 (IDH-1) (Dianova, Hamburg, Germany; 1 in 20 dilution) was performed using a standard protocol employing an automated IHC system (Bond Max, Leica Biosystems, Milton Keynes, UK).

Histological examination revealed a non-encapsulated, infiltrative brain mass. The tumour was separated into irregular, sometimes confluent lobules, which were divided by, and surrounded by, cerebral neuropil. Within the lobules, the neoplastic cells were arranged in more or less solid sheets supported by variably thick bands of collagen. There were multifocal prominent microvascular proliferations creating vascular loops or glomerular-like structures arranged in lines or clusters, with notable palisading of the tumour cells bordering these structures (Fig. 1). Neoplastic cells were polyhedral with pale, vacuolated or finely granular cytoplasm. Nuclei were round to irregularly shaped and were moderately hyperchromatic with finely to coarsely stippled chromatin and small, frequently indistinct, basophilic nucleoli. Mitoses were frequent, with up to 20 mitotic figures per 10 high-power fields (per 2.37 mm^2). There was a moderate degree of cellular pleomorphism, anisocytosis and anisokaryosis. Scattered, variably sized foci of necrosis were present multifocally and comprised an estimated 35% of the tissue area of the mass examined.

While the adjacent neuropil exhibited the expected expression of GFAP (positive internal control tissue), the majority of the neoplastic cells did not express this molecule, with only thin cytoplasmic processes exhibiting intense labelling (Fig. 2). The majority of the neoplastic cells did not show positive labelling for Olig2, although expected labelling was observed in adjacent tissue. The majority of the neoplastic cells had intense nuclear expression of ATRX (Supplementary Fig. 2). The neoplastic cells did not express an IDH mutation specific to R132H substitution in isocitrate dehydrogenase-1. Approximately 20% of the neoplastic cells had intense nuclear expression of Ki67 (Supplementary Fig. 3). The histological appearance of the neoplastic cells and the immunohistochemical labelling were considered to be most consistent with a diagnosis of a glioblastoma.

Case 2, a female, wild caught, aye-aye estimated as 26 years old was humanely destroyed due to persistent difficulties in locomotion suspected to be related to previously diagnosed spondylosis affecting lumbar vertebrae. Post-mortem examination revealed 225 ml of serosanguineous fluid in the abdomen. The most significant changes were observed in the liver, where the hepatic parenchyma in the central

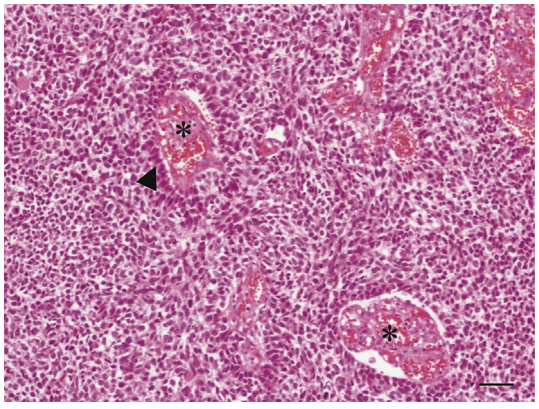

Fig. 1. Glioblastoma in an aye-aye. Neoplastic cells are arranged in densely packed sheets and a palisade (arrowhead) around prominent 'glomeruloid' microvascular proliferations (asterisk). HE. Bar, 100 µm.

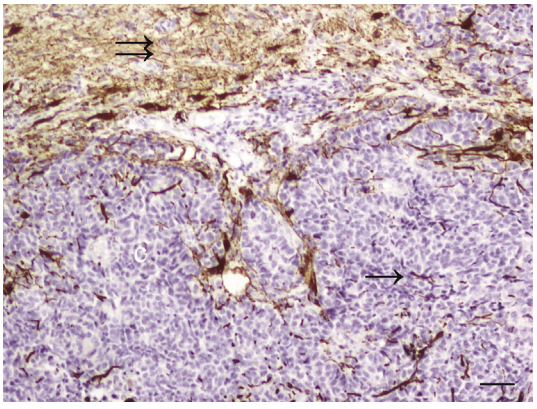

Fig. 2. Glioblastoma in an aye-aye. Adjacent neuropil shows expected expression of GFAP (positive internal control; double arrows), but the majority of neoplastic cells do not show GFAP labelling, with only thin cytoplasmic processes exhibiting intense positive expression (arrow). IHC. Bar, 100 µm.