Neck Kaposiform haemangioendothelioma in a Fischer's lovebird (Agapornis fischeri)

NECK KAPOSIFORM HAEMANGIOENDOTHELIOMA IN A FISCHER'S LOVEBIRD (AGAPORNIS FISCHERI)

Running head: Kaposiform haemangioendothelioma in a parrot

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ABSTRACT

A six-year-old female Fischer's lovebird (Agapornis fischeri) presented at necropsy with a cutaneous mass on the neck, 3,5 cm in diameter, yielding and with blood content. Histopathological findings showed a neoplasm characterized by proliferation of vascular endothelial cells. The histology of the mass revealed a multinodular, focally infiltrating tumor. Deeper dermal nodules were made of spindle cells forming vascular slits reminiscent of the histology seen in Kaposi's sarcoma (KS). More superficially located dermal nodules consisted of small blood vessels, with histology resembling capillary hemangioma. The spindle cells and capillaries were strongly positive for vimentin, endothelial cell marker CD31, and negative for sarcomeric α-smooth muscle actin (α-SMA). Intravascular platelet trapping and Periodic acid-Schiff (PAS)-positive hyaline globules were also observed. Differential diagnosis included Kaposi's sarcoma, capillary haemangioma, spindle cell haemangioendothelioma, and epithelioid haemangioendothelioma. Based on morphological and immunohistochemical findings, the tumor was diagnosed as a cutaneous Kaposiform haemangioendothelioma (KHE), a rare, low-grade malignant vascular neoplasm. Other organs showed no abnormalities. PCR amplifications, conducted using Kaposi's sarcoma-associated herpesvirus (KSHV)-specific primers and degenerate sets of primers designed to detect and characterize members of the Herpesviridae, on DNA extracted from tumor tissue and from whole blood failed to amplify any KSHV-related sequence. Moreover, no specific signal was obtained using primers for detection of psittacine herpesvirus, known to be linked to Pacheco's disease in parrots. To the best of our knowledge, this unusual case is the third report of KHE in a non-human animal species, the first described in a bird.

KEY WORDS: Agapornis fischeri; Fischer's Lovebird; Herpesvirus; Kaposiform hemangioendothelioma; Kaposi's sarcoma; vascular tumor.

SHORT COMMUNICATION

Kaposiform hemangioendothelioma (KHE) is a rare locally aggressive vascular tumor of the skin, deep soft tissue, and bone in children, characterized by infiltrating nodules and sheets of spindle cells, and unmistakable resemblance to Kaposi's sarcoma (KS), first described in 1993 by Zukerberg (Zukerberg et al., 1993; Tsang, 2002). The name was coined for its distinctive morphology, characterized by a Kaposi's sarcoma-like spindle cell growth pattern. Clinically, the tumor shows the predilection for involving the retroperitoneum, mediastinum, and deeper soft tissues of the neck (Mentzel et al., 1997; Chung et al., 2003; Lyons et al., 2004; Sun et al., 2006), trunk and extremities (Tsang, 2002). Some lesions were reported in conjunction with lymphangiomatosis or were associated with the Kasabach-Merritt syndrome (KMS) (Fukinaga et al., 1996). These tumors are usually locally aggressive, and distant metastases have not been reported yet. KS, considered a variant of hemangiosarcoma, does not normally occur in animals. Until now, Kaposi-like sarcoma was only described in a few cases, involving mammal species. The first case regarded a mice injected with 1,2-dimethylhydrazine dihydrochloride (Sato et al., 1986). The tumor was shown to develop after chemical induction only in the liver and not in the skin (Sato et al., 1986). Another case of low-grade malignant vascular neoplasm classified as epithelioid hemangioendothelioma, was also reported in the lung of a dog (Machida et al., 1998) and the lesion was characterized by tumor cells with abundant eosinophilic cytoplasm and the presence of intracytoplasmic vacuoles. The last case classified as KHE was described in a 10-year-old male dog. This tumor, located on the ventromedial surface of the posterior limb, consisted microscopically in a multinodular mass with sheets of spindled endothelial cells forming vascular slits similar to KS and peripheral tumor lobules resembling capillary hemangioma (Vincek et al., 2004). A very similar tumor, called Kaposi-like vascular tumor, has previously been described in both the latest World Health Organization fascicle on the Histological Classification of Mesenchymal Tumors of Skin and Soft Tissues of Domestic Animals, published in 1998, and in Tumors in Domestic Animals (Hendrick, 2002). In this description, the Author proposed calling the

lesion ‘‘Kaposi-like vascular tumor’’ because the tumor has ‘‘features of KS and KHE of humans’’(Hendrick, 2002). The only case of a KS-like vascular tumor associated with the infection of a KSHV homolog is represented by the macaque retroperitoneal fibromatosis, a vascular fibroproliferative malignancy with morphological and histological similarities to KS (Rose et al., 1997). A simian homolog of KSHV was identified in retroperitoneal fibromatosis lesions of two macaque species using a consensus degenerate hybrid oligonucleotide primer (CODEHOP) technique (Van Devanter et al., 1996; Rose et al., 1997).

To the best of our knowledge, we reported here for the first time a case of a Kaposi-like vascular tumor occurring in a bird.

A case of KHE involving the anterior neck, not associated with KMS despite its size, in a 6-year old female Fischer’s lovebird (Agapornis fischeri) has been described. The parrot was brought for necropsy to our laboratory with an evident sub-cutaneous mass on the neck, well-delimited, reddish-black in color, with abundance of blood clots on cut surface, 3,5 cm in diameter, yielding and with blood content (Figure 1). The breeder reported that the bird was kept in an aviary with other lovebirds, and he noticed the tumefaction on the neck from 2 months before death; no other symptoms were identified from the owner. There were no signs of tracheal compression, and the mass did not appear to invade the hypoglottic area and floor of mouth in the intra-oral examination. No lesions were seen macro- and microscopically in the other organs of the parrot. The mass was entirely removed and used partially for molecular tests and partially fixed in 10% buffered formalin for routine histological examination. Three-micrometer-thick sections from paraffin embedded neoplastic tissue were stained with haematoxylin-eosin (HE) and Periodic acid Shift (PAS) stain for histopathological examination (Figure 2 A). To characterize immunohistochemically the tumour, immunostaining was also performed by using the following antibodies: Vimentin (1:200, DAKO), CD31 (1:200, DAKO), sarcomeric α-smooth muscle actin (α-SMA) (1:100, 1A4, DAKO), and factor VIII-related antigen (FVIIIRAg, 1:200, DAKO). On the basis of the aetiological association of KS development with KSHV infection in humans, the presence of herpesvirus DNA was sought

in the neoplastic tissue. Briefly, DNA was extracted from the neck mass and 91 whole blood samples taken from the internal part of the tumor using a commercial DNA isolation kit (DNeasy Blood & Tissue Kit, Qiagen, Milan, Italy), according to the manufacturer's instructions. Extracted DNA was quantified and stored at -80°C until use. The amplificability of DNA samples was tested using oligonucleotide primers designed to amplify a conserved segment of the mitochondrial DNA cytochrome b gene of the avian species (Tramuta et al., 2006). To assess the presence of KSHV, DNA extracted from the tumor and whole blood was analyzed by PCR using ORF26-specific and ORF25-specific primers whose amplification conditions and sensitivity have previously been described (Calabrò et al., 1999). To possibly identify an avian homolog of KSHV, a refined technology using optimized DNA polymerase (CODEHOP) primers was used (Rose et al., 2003). Moreover, the presence of an avian herpesvirus normally recognized as being responsible for diseases in parrot (Pacheco's disease) was also investigated to exclude its involvement, using specific primers and conditions previously described (Tomaszewski et al., 2001). As clinical signs, the latter could cause papillomatosis in the pharynx, even if it is not its most frequent feature.

Morphologically, the tumor consisted of dense spindle cells with a nodular growth pattern, and with hypocellular areas of hyalinized fibrous stroma (Figure 2 A). The spindle tumor cells showed no cytological atypia, and focally exhibit slit-like and gaped lumen, but most often did not show a luminal formation. The spindle tumor cells may appear epithelioid with glomeruloid capillary proliferation and formation of micro thrombi (Figure 2 B). More superficially, the neoplasm consisted of small blood vessels, with histology resembling capillary hemangioma. The tumor cells, whether epithelioid or spindled were immunoreactive to Vimentin (Figure 2 B), CD31 (Figures 2 C and D), but not to sarcomeric α-smooth muscle actin (α-SMA), and factor VIII-related antigen, in contrast to well-formed capillaries and mature vessels (data not shown). In areas, few lymphocytes but not plasma cells were seen. There was no encapsulation, and the tumor infiltrated the peripheral skeleton muscles. Large feeding vessels were present at the periphery of the tumor. The solid area of spindle cells associated with slit-like lumen containing red blood cells were reminiscent of KS.

Intravascular platelet trapping and PAS–positive hyaline globules were also seen (Figure 2 A). In a differential diagnosis, the presence of spindle cells with slit-like channels was not suggestive of capillary hemangioma. Moreover, intracytoplasmic vacuoles containing red blood cells and the cavernous vascular spaces, usually present in the epithelioid hemangioendothelioma and in spindle cell hemangioendothelioma, respectively, were not observed. Finally, lymphoplasmacytic infiltrate and a more diffused infiltrative pattern, typically indicative of KS, were not evidenced.

PCR analyses performed to detect KSHV and KSHV-like sequences did not reveal the presence of KSHV-related products in the neoplastic tissue. Moreover, PCR analyses carried out to detect Pacheco's disease virus failed to detect specific sequences. DNA extracted from whole blood was also analyzed and gave negative results as well. On the basis of these findings, the diagnosis of KHE or Kaposi-like vascular tumor was given.

KHE has features common to both capillary hemangioma and KS (Brasanac et al., 2003). It has generally been considered distinct from other vascular neoplasms. This type of vascular neoplasia, extremely rare in animals, should be classified as ''Kaposi-like vascular tumor'' according to Hendrick's suggestions (2002). Our case, the first described in a bird, involved the neck region, in a similar pattern as reported also in children (Sun et al., 2006). Despite its rarity, the parrot's tumor showed rather typical morphology of KHE, with a deeply infiltrative nodular growth, dense fibrous septa, spindle cells with slit-like vascular lumen and unmistakable resemblance to KS (Zukerberg et al., 1993; Hu et al., 1998; Tsang, 2002). In our case, both epithelioid and spindle tumor cells expressed endothelial marker CD31, but not FVIII-Rag, results consistent with the reported observations (Tsang, 2002; Lyons et al., 2004). As CD31-positive spindle cells in KS lesions usually also express lymphatic endothelial markers such as podoplanin and VEGFR-3 (Weninger et al., 1999; Pires et al., 2009), the availability of antibodies recognizing these markers in avian tissues would greatly improve the diagnostic accuracy. Mature capillaries and vessels in this parrot were positive for FVIII-Rag, and □-SMA was expressed by pericytes that outlined tumor spindle cells. KHE was classified as borderline malignant because of its locally aggressive behavior, causing

significant morbidity and mortality as a result of the compression and invasion of surrounding structures, depending on the size, anatomic site, and extent of the neoplasm (Zukerberg et al., 1993; Mac-Moune et al., 2001; Lyons et al., 2004). Only local, but no distant metastasis has been reported (Lyons et al., 2004). As in our case, and also in human reports, three of 21 cases with neck involvement died, with death related to disease complications rather than to tumor recurrence (Sun et al., 2006). Although KS has been associated with KSHV infection, this virus has never been found in association with KHE (Martinez et al., 2004). In our case, a refined technique, designed to amplify novel members of Herpesviridae, was performed on DNA extracted from the neoplastic tissue. This assay was proven to be quite robust and has been used to amplify more than 30 previously unknown herpesviruses from members of the alpha, beta and gamma subfamilies (Rose, 2005). Although we cannot exclude the presence of distantly related herpesviruses, KSHV-like sequences were not evidenced. Similarly to what has been reported in non-human mammals (Vincek et al., 2004), the unusual tumor was found in a relatively older animal.

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CONFLICT OF INTEREST

None.

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259

260 FIGURES

261 Figure 1: Fischer’s lovebird (Agapornis fischeri); cutaneous mass on the neck.

262 Figure 2: A) Higher magnification showing spindle cells, including group of cells forming micro
263 vessels and containing RBCs. Eosinophilic and PAS positive bodies amid spindle shaped vascular
264 cells were also seen (arrow heads). (PAS stain; scale bar= 20μm). B) The tumor cells, whether
265 epithelioid or spindled, were immunoreactive to Vimentin (arrow heads) (anti-Vimentin IHC stain;
266 scale bar = 100 μm). C) Immunohistochemistry for CD31 highlights small, slit-like vascular spaces;
267 note the positive spindle tumor cells (small arrow heads) and that appear epithelioid with
268 glomeruloid capillary proliferation (large arrow heads). (anti-CD31 IHC stain; scale bar = 250μm).
269 D) Nodules of infiltrating epithelioid cells (arrow head) showing immunoreactivity to CD31. (Anti-
270 CD31 IHC stain; scale bar = 100 μm).