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Case Report
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Fibrosarcoma of the eyelid in two sibling Czech wolfdogs

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Abstract
Most canine tumors of the eyelid are tumors generally encountered in the skin. They are most commonly of epithelial origin and benign. In this report, we describe the cases of two sibling Czech wolfdogs presented, one year apart, with a subcutaneous mass involving the left eyelid. Both lesions were histologically consistent with a diagnosis of subcutaneous fibrosarcoma. Immunohistochemical analyses of the tumors revealed a mild positivity for vimentin and negativity for GFAP, desmin, αSMA, myoglobin, S100, PNL2 and calponin, excluding all differential diagnosis (i.e. peripheral nerve sheath tumor, melanoma, perivascular sarcoma, myofibroblastic sarcoma, rhabdomyosarcoma). To the best of authors' knowledge, this is the first report of canine eyelid fibrosarcoma. Since this rare tumor has been observed in two full siblings, we could speculate the existence of some genetic predisposition to sarcoma, however the present data did not allow any definite conclusion on the etiopathogenesis or genetic basis of these tumors.
Keywords: Dog, Eyelid tumor, Sarcoma, Siblings.

Introduction

Most tumors affecting canine eyelids are tumors generally encountered in the skin. They include melanocytic tumors, sebaceous gland adenomas, histiocytic and mast cell tumors, squamous papillomas and carcinomas, trichoblastomas and trichoepitheliomas (Krehbiel and Langham, 1975; Dubielzig, 2002). Benign tumors are more common than malignant ones, the latter being rare and usually not metastasizing, and epithelial tumors are considered more common than mesenchymal ones (Krehbiel and Langham, 1975). In the present report the authors described two unusual cases of mesenchymal tumors of the eyelids (fibrosarcomas) presenting in two sibling Czech wolfdogs.

Case details

Case 1

A 10-year-old male spayed Czech wolfdog was presented to a private veterinary practice in November 2014 for a bulging on the lower lid of the left eye. The owners reported that the lesion had grown over several months and currently caused a slight closure of the palpebral fissure. There was no history of trauma, of previous ocular or systemic health problems.

Menace responses, palpebral reflexes, dazzle and direct and consensual pupillary light responses were present in both eyes (OU). Ophthalmic examination, slit-lamp biomicroscopy, indirect ophthalmoscopy, and applanation tonometry were carried out under general anesthesia due to the aggressive behavior of the dog.

In the lower left eyelid, a subcutaneous mass, not ulcerated and not adherent to the skin, was detected, causing mild epiphora and mild conjunctival hyperemia OS (left eye).

The cornea was fluorescein stain negative OU, intraocular pressure (IOP) was within normal limits and fundus examination was normal.

A skull x-ray and an ultrasound of the mass and of the abdomen were performed as ancillary tests. A skyline projection showed that the orbital bone was not affected. At ultrasound the mass was dense and mildly vascularised. Thoracic X-ray and abdominal ultrasounds were unremarkable.

Complete blood count and serum chemistry results, included as pre-operative diagnostics, were within normal limits.

The mass, which rested on the orbital bone, without infiltrating it, was surgically removed. Nine months later (August 2015), the dog presented to emergency with severe hemoperitoneum due to rupture of a splenic hematoma. The dog was humanely euthanized. No recurrence of the eyelid mass was recorded at that time. Necropsy was proposed but declined by the owner.

Case 2

A 11-years-old female Czech wolfdog, sibling of case 1, was presented in October 2015 with a bulging in the left eye lower eyelid causing deformation of the eyelid profile (Fig. 1A).

At ophthalmic examination, carried out using an E-collar due to the aggressive behavior of the dog, menace responses, palpebral reflexes, dazzle and direct and consensual pupillary light responses were present.

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A large subcutaneous mass, not ulcerated and not adherent to the skin, causing closure of the palpebral fissure was present in the lower eyelid OS. Other investigations (slit-lamp bio microscopy, indirect ophthalmoscopy, and applanation tonometry) were carried out under general anesthesia and the findings were within normal limits.

Abdominal ultrasounds were performed and a small splenic nodular lesion was detected. FNA cytology of the splenic lesion was consistent with splenic hematoma. Complete blood count and serum chemistry panel were within normal limits.

The eyelid mass was surgically removed and submitted for histology.

In February 2016 the dog showed recurrence of the eyelid neoplasia, presenting at this time as a large mass extending to the orbit and causing exophthalmos. At ultrasound examination, compression and distortion of the eye globe without scleral invasion were observed. Complete blood count and serum chemistry panel were within normal limits and clinical staging was negative. Orbital exenteration was surgically performed, and all tissues removed were submitted for histology.

In June 2016 the dog presented with a further recurrence of the tumor within the orbital cavity, with swelling of the eyelid suture, and with difficult mouth opening. Due to the severe deterioration of general conditions, the owner elicited for euthanasia. Necropsy was not accepted.

Histopathology

All samples were fixed in 10% buffered formalin and routinely processed for histology. Microtomic section were obtained and stained with hematoxylin and eosin for histopathological examination.

In case 1, a 2.5 cm bilobate expansile subcutaneous mass, partially circumscribed by a fibrous capsule and focally extending to the cut borders, was observed. The neoplasia had two distinct cell populations with different growth patterns. The first component consisted of large interlacing bundles of amorphous fibrillar material (collagen) with scarce interspersed spindle cells characterized by mild atypia and less than 1 mitosis in 10 HPF.

The second component consisted of long, irregular, densely cellular bundles of spindle cells with indistinct borders, oval vesicular nuclei with marginated chromatin and scant eosinophilic cytoplasm. Anisocytosis and anisokaryosis were moderate and mitoses ranged from 0 to 3 per HPF (mitotic activity index 0.7) (Fig. 2). A large necrotic center and hemosiderin deposits were also observed. A diagnosis of subcutaneous fibrosarcoma (grade 2) was posed. Differential diagnosis included poorly differentiated peripheral nerve sheath tumor (PNST), perivascular wall tumor (PWT), myofibroblastic sarcoma, amelanotic melanoma and rhabdomyosarcoma.

In case 2, a bilobate neoplastic mass infiltrated the eyelid subcutaneous tissue. The neoplasia was partially enclosed by a pseudocapsule, and, where the capsule lacked, infiltrated muscular layers and extended to the cut borders. Neoplastic cells were spindle-shaped, arranged in interlacing bundles or occasionally in whorls circumscribing blood vessels and were characterized by indistinct cell borders, high nuclear/cytoplasmic ratio, scarce eosinophilic cytoplasm with occasional vacuolation, and oval nucleus with finely granular chromatin and one or two small nucleoli.

Anisocytosis and anisokaryosis were moderate and mitoses ranged from 0 to 4 per HPF (mitotic activity index 1.7). Large multifocal areas of necrosis were also present. A diagnosis of poorly differentiated subcutaneous fibrosarcoma (grade 3) was posed. Differential diagnoses considered were the same as listed for case 1.

Recurrence of neoplasia in case 2 was a 6,5 cm mass expanding the subcutaneous tissue and invading skeletal muscles, adipose tissue and salivary glands (Fig. 1B).

The neoplasia was densely cellular, poorly demarcated and un-encapsulated, with cells variably arranged in long interwoven bundles, whorls or herringbone. Cells were spindle-shaped with moderate fibrillary cytoplasm and oval nuclei with grossly granular chromatin and no evident nucleoli. Anysocytosis and anysokaryosis were moderate and mitoses ranged 0 to 1 per HPF (mitotic activity index 0.1).

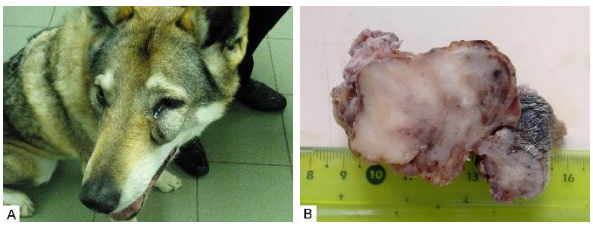

Fig. 1. (A): External view of the neoplastic mass *in situ,* case 2 at first presentation. **(B):** Longitudinal section of the formalin-fixed mass, case 2 recurrence.

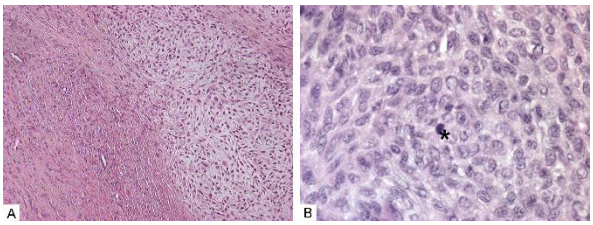

Fig. 2. (A): Fibrosarcoma composed by two cellular populations consisting in large interlacing bundles of amorphous fibrillar material with scarce interspersed spindle cells (on the right) and long densely cellular bundles of spindle cells (on the left) (H&E, 10X). **(B):** Neoplastic cells exhibited moderate anisocytosis and anisokaryosis. A mitotic figure is present (*) (H&E, 40X).

Multifocal hemorrhages and deposits of hematoidin pigment were also present. A diagnosis of subcutaneous fibrosarcoma (grade 2) was posed. The eye globe was unremarkable, characterized by diffuse blood vessels hyperemia and a small aggregate of mature lymphocytes in the episcleral area adjacent to the limbus.

Immunohistochemistry

Serial microtomic sections of all tumors were obtained, mounted on polylysine coated slides (Menzel-Gläser, Braunschweig, Germany) and immunostained with the standard ABC method using a panel of monoclonal and polyclonal antibodies. Details of antibodies used, dilutions, retrieval methods and positive controls are listed in Table 1. DAB (3,3'-diaminobenzidine) or AEC (3-amino-9-ethylcarbazole) substrate-chromogen kit (Vector Laboratories, Burlingame, USA) were used as chromogen, sections were counterstained with Mayer's hematoxylin. Negative controls were prepared by replacing the respective primary antibody with normal rabbit or mouse serum (non-immune serum, Dakocytomation).

Consistent immunohistochemical results were obtained in all tumors (case 1, case 2, case 2 recurrence): in all cases, neoplastic cells were moderately, diffusely, intracytoplasmically labelled with vimentin (Fig. 3). GFAP, desmin, αSMA, myoglobin, S100, PNL2 and calponin were always negative. Specifically PNL2 and S100 negative staining excluded melanocytic origin; desmin, αSMA, myoglobin and calponin negativity excluded myofibroblastic sarcoma, PWT and rhabdomyosarcoma, and S100 and GFAP negativity excluded PNST. On this basis, the diagnosis of fibrosarcoma was confirmed.

Discussion

This case report describes the clinical and histopathological features of eyelid fibrosarcoma occurring in two full sibling Czech wolfdogs. To the best of the authors' knowledge, this is the first report of this type of eyelid tumor in the canine species and the first report describing the occurrence of eyelid fibrosarcoma in sibling dogs.

Canine eyelid sarcomas are infrequent: generally, eyelid epithelial neoplasms outnumber the mesenchymal ones by a ratio of 5 to 1 and benign neoplasm outnumber malignant ones by a ratio of 3 to 1 (Stades and van der Woerdt, 2013). The tumors described in this case report presented as subcutaneous eyelid masses that were histologically consistent with a diagnosis of fibrosarcoma, characterized respectively by an intermediate or high grade of morphological malignancy (grade 2 and 3).

An aggressive behavior was confirmed in case 2 by the early recurrence of the lesion. Immunohistochemical staining excluded poorly differentiated forms of neurogenic, muscular and melanocytic neoplasia. In dogs, palpebral fibrosarcoma has not been reported so far.

Recently two cases of periocular extracranial cutaneous meningiomas have been reported. Eyelid meningiomas exhibited spindle to epithelioid cells, and were characterized by lobular arrangement and positivity to S100 immuno-labelling (Teixeira *et al.*, 2014). Meningioma was not initially considered among our differentials, however S100 immunohistochemical staining was consistently negative in all our samples, excluding a possible meningeal origin of neoplastic cells in our cases.

Table 1. Immunohistochemical examination: details of antibodies used, dilutions, retrieval methods and positive controls.

IHC marker	Antigen retrieval	Primary antibody	Positive control
Vimentin	Microwave oven, citrate buffer pH 6.0 (10', 500W)	Clone 3B4; dilution 1:1000, Dako, Carpinteria, USA	Internal: dermal fibrocytes
Desmin	Pepsin enzymatic digestion*	Clone NCL-L-DES-DERII dilution 1:150, Leica Biosystem, Nussloch, Germany	Internal: muscle of arterial wall
αSMA	None	Clone 1A4, dilution 1:2000, Dako, Carpinteria, USA	Internal: muscle of arterial wall
Myoglobin	None	Polyclonal, dilution 1:10, Dako, Carpinteria, USA	Internal: skeletal muscles
GFAP	None	Polyclonal, dilution 1:3000, Dako, Carpinteria, USA	Internal: peripheral nerves
PNL2	Microwave oven, EDTA buffer pH 8.5 (10', 500W)	Clone PNL2, dilution 1:50, Monosan, Uden, Netherlands	Section of canine melanoma
S100	None	Polyclonal, dilution 1:100, Dako, Carpinteria, USA	Internal: peripheral nerves
Calponin	Proteinase K (37°C 10') + Microwave oven, citrate buffer pH 6.0 (10', 500W)	Clone hCP, dilution 1:2000, Sigma-Aldrich, Saint Louis, MI, USA	Internal: muscle of arterial wall

*Digest-All Invitrogen, Thermo Fisher Scientific, Carlsbad, USA.

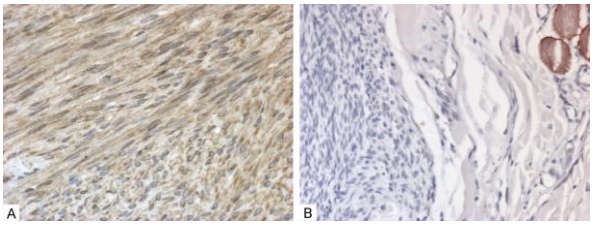

Fig. 3. (A): Immunohistochemistry anti-vimentin, intracytoplasmic positivity of neoplastic cells (DAB chromogen, 40X). **(B):** Immunohistochemistry anti-desmin, negativity of neoplastic cells (on the left) with positive skeletal muscle as internal control (AEC chromogen, 20X).

Most reports of canine eyelid sarcoma in the literature at a closer view are actually extension of orbital sarcomas presenting as eyelid swelling. For example, orbital embryonal rhabdomyosarcoma, typically diagnosed in young patients, may clinically presents as eyelid enlargement but it should be considered a primary orbital tumor (Plowman, 2007; Kato *et al*., 2012).

In our cases initial presentation was restricted to the eyelid subcutis, without orbital involvement. Moreover, markers of muscle differentiation were always negative in the present cases.

Although not previously described in the literature in this anatomic location, based on histological features observed, canine perivascular wall tumors (specifically angioleiomyosarcoma) was also considered as a possible differential diagnosis for the tumors described in the present report. Angioleiomyosarcoma can be negative to αSMA immune-labelling, but they are positive for calponin staining (Avallone *et al*., 2007). The immunohistochemical staining for αSMA and calponin were both negative in our cases and these results excluded the perivascular origin of the tumors.

Eyelid sarcomas are also rare in species other than dog. In man, palpebral angiosarcoma, Kaposi's sarcoma and malignant peripheral nerve sheath tumor have been described (Pe'er, 2016). Palpebral lymphangiosarcomas and hemangiosarcomas have been reported in horses (Serena *et al*., 2006; Gerding *et al*., 2015), liposarcoma in guinea pigs (Quinton *et al*., 2013), hemangiosarcomas and peripheral nerve sheath tumors in cats (Newkirk and Rohrbach, 2009).

Interestingly, the two dogs presented in this case report were full-siblings with lesions similar in location, gross and histological morphology.

In human medicine there are proved evidences of tumors arising on genetic bases. Different inherited genetic syndromes increase the risk for sarcoma development, such as neurofibromatosis (NF1), Li-Fraumeni syndrome (LFS), and Retinoblastoma (Rb) (Burningham *et al*., 2012; Thomas *et al*., 2012). NF1 derives from an autosomal dominant event and increases the risk of developing malignant peripheral nerve sheath tumor (Evans *et al*., 2012); LFS results from germline mutations in the tumor suppressor gene *TP53* and it is strongly related to the early development of a wide variety of tumors (eg., breast cancer, soft tissue sarcoma, brain tumor, adrenocortical carcinoma) (Gonzalez *et al*., 2009); Rb leads to a greater risk of developing secondary tumors, particularly osteosarcoma (Wong *et al*., 1997).

In the veterinary literature there are sparse reports of tumors affecting littermates (Teske *et al*., 1994; Shaw *et al*., 2010; Munday *et al*., 2012), in which the role of an undetermined underlying genetic predisposition has been hypothesized, and few studies have investigated the possible genetic risk factors in carcinogenesis, like a recent wide-genome study in canine mammary tumors (Melin *et al*., 2016).

The available data regarding the two Czech wolfdogs described in the present report and the current knowledge are not sufficient to speculate of a genetic bases underlying the etiopathogenesis of these sarcomas. However, the occurrence in two full-sibling dogs of exceedingly uncommon eyelid fibrosarcomas, similar for location, age of onset, clinical and pathological features, leads to hypothesize that carcinogenesis may have been influenced by shared undetermined genetic and environmental factors,.

The study of familial tumors in dogs is a field of interest that would be worth of deeper investigations.

Conclusion

To the best of authors' knowledge this is the first report of fibrosarcoma of the eyelids in the canine species. Moreover eyelid fibrosarcomas in the present report were observed in two full-sibling dogs, leading to the speculation that a possible genetic factors may played a role in the carcinogenesis of these tumors.

Conflict of interests

The Author declare that there is no conflict of interest.

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