

Hemangiosarcoma of the third eyelid in a horse

Hemangiosarcoma van het derde ooglid van een paard

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ABSTRACT

A 12-year-old Belgian Warmblood mare was referred to the equine clinic of the Faculty of Veterinary Medicine (Ghent University) for a mass on the third eyelid of the right eye. The horse had been having a recurrent red-tinged ocular discharge for several months. After complete ophthalmologic examination, a large part of the third eyelid was surgically excised and submitted for histopathologic examination. The histopathologic examination revealed a proliferation of blood-filled vascular spaces lined by a single layer of flattened cells, which were identified as endothelial cells using immunohistochemistry. The tumor was identified as a cavernous low-grade hemangiosarcoma. No complications were reported six months after surgery.

SAMENVATTING

Een 12-jarig Belgisch warmbloedpaard werd doorgestuurd naar de paardenkliniek van de Faculteit Diergeneeskunde (Universiteit Gent) voor een massa aan de membrana nictitans van het rechteroog. De merrie had sinds enkele maanden een terugkerende roodkleurige vochtscheiding uit dit oog. Na een volledig oftalmologisch onderzoek werd een groot deel van het derde ooglid chirurgisch verwijderd en onderworpen aan een histopathologisch onderzoek. Hierbij werd een proliferatie van met bloed gevulde vasculaire ruimten gevonden. De ruimten waren afgelijnd met afgeplatte cellen die immunohistochemisch geïdentificeerd werden als endotheelcellen. De diagnose van caverneus, laaggradig hemangiosarcoma werd gesteld. Zes maanden na de ingreep werden geen complicaties opgemerkt.

INTRODUCTION

Spontaneous tumors of blood vessel endothelial cells have commonly been described in the dog, less frequently in the cat and horse, and sporadically in most other domestic species (Goldschmidt *et al.*, 2002; Poulsen *et al.*, 2008).

Hemangiosarcoma is a malignant tumor of the endothelial cells. It may be classified as being either of the capillary or of the cavernous type, depending on the size of the vascular channels formed. The degree of differentiation is extremely variable, ranging from well-differentiated tumors with clear vascular channels to poorly differentiated tumors with minimal lumen formation (Jubb *et al.*, 2007). The cause of most hemangiomas and hemangiosarcomas is unknown. In humans, these tumors have been associated with exposure to thorium dioxide, arsenicals, and vinyl chloride (Jubb *et al.*, 1993). It appears that chronic solar damage on lightly pigmented, sparsely haired dogs may also contribute to the development of cutaneous hemangiomas (Hargis *et al.*, 1992). The solar impact

was also suggested to be a risk factor for conjunctival hemangiomas and hemangiosarcomas in dogs (Pirie *et al.*, 2006).

Ocular hemangiomas and hemangiosarcomas can affect the conjunctivae, the nictitans membrane and the eyelids (Lavach *et al.*, 1977). They may spread further into the globe, the orbit, the local lymph node and even into the facial muscles (Hacker *et al.*, 1986).

The present case report describes an ocular hemangiosarcoma involving the third eyelid in a horse.

CASE REPORT

History

A 12-year-old Belgian Warmblood mare was referred with a complaint of recurrent mild red ocular discharge and a 'red medial corner' in the right eye during the preceding month. There was no history of previous eye problems. Topical treatment with oxytetracyclin / polymixin B (Terramycin®, Pfizer, Belgium) followed by dexamethasone/neomycin-poly-

myxine (Maxitrol®, SA Alcon-Couvreur NV, Belgium) resulted only in a minimal improvement of the symptoms. An increase in red discharge was noted a week after the beginning of this topical treatment, and the horse was then referred for further treatment.

Clinical examination

The horse was in good general condition. A small amount of blood was noted in the medial corner of the right eye, as a result of which the nictitans membrane was hard to visualize.

The horse was sedated with romifidine (80ug/kg, IV, Sedivet®, Boehringer Ingelheim, Germany) and butorphanol (20ug/kg, IV, Dolorex®, Intervet, Belgium), followed by a block of the auriculopalpebral nerve with mepivacaine (7ml of Scandicaïne 2 %®, Astra Zenica, Belgium) and desensitization of the cornea by topical administration of oxybuprocain HCl (0.5 ml Unicaïne® 0,4%, Thea, Belgium).

Both eyes had normal pupil and menace reflexes, with the left eye showing no abnormalities. The medial canthus of the right eye had some remnants of the serosanguinous discharge. A lobulated, red fragile mass of approximately 2 cm diameter with craterlike lesions was noted on the outer surface of the nictitans membrane. The nasolacrimal duct was patent and fluorescein staining failed to show corneal lesions. The differential diagnosis included: tumors such as squamous cell carcinoma, epibulbar melanoma, intravascular papillary endothelial hyperplasia and non-neoplastic lesions such as inflammatory granulation tissue. Upon consent of the owner, surgical removal of the nictitans membrane was performed.

Surgery

The anaesthetized horse was placed in left lateral recumbent position. An auriculopalpebral nerve block was performed with mepivacaine (8 ml Scandicaïne 2 %®, Astra Zenica, Belgium). The surgical site was shaved, scrubbed and disinfected and the right cornea was desensitized with topical application of oxybuprocaine HCl (0.5 ml Unicaïne 0.4 %® Thea, Belgium) and protected with a gentamycin based gel (Clinigel-Vet®, Janssen Animal Health, Belgium). A diluted epinephrine solution was administered on the nictitans membrane (4 ml epinephrine 1% into 16 ml NaCl 0.09%) to limit possible bleeding during surgery. The nictitans membrane was exteriorized towards the lateral canthus using a 2/0 polyglactin single (Vicryl, Johnson-Johnson) suture. The third eyelid was almost completely excised using electrocauterization (Erbe ICC 300, Georgia, USA), whereafter the remaining bulbar and palpebral conjunctivae of the nictitans membrane were sutured with a simple continuous pattern using a 4/0 polyglactin 910 (Vicryl, Johnson-Johnson). The mass was fixed in formalin (10%) and submitted for histological examination. The horse received flunixin meglumine (1.1mg/kg /d, Fyna-

dine® Paste, Shering Plough, Spain) for 2 days; no local treatment was administered.

Histology

The formalin-fixed tissue was processed, embedded in paraffin wax, sectioned at 4µm, stained with hematoxylin and eosin (HE), according to standard techniques and microscopically examined.

Immunohistochemistry

Paraffin embedded tissues were sectioned at 4µm, mounted on coated slides (SuperFrost®Plus, Menzel-Gläser, Braunschweig, Germany), and allowed to dry for 1 hour at 60°C and then overnight at 37°C. Thereafter the sections were deparaffinized and incubated with mouse monoclonal anti-human Ki67, which is a cellular marker for proliferation (Novocastra, Newcastle, United Kingdom; 1 in 50) and polyclonal rabbit anti-human *von Willebrand* factor (vWF, also known as Factor VIII-related antigen, a marker for endothelial cells) (Dakocytomation, DK-2600 Glostrup, Denmark; 1/200). A standard immunohistochemical avidin biotin complex method with diaminobenzidine as chromogen was used (Envision, Dakocytomation). Samples of spleen and large intestine, used as positive controls, were run concurrently for both of the antibodies tested.

Results

Macroscopic evaluation of the right nictitans membrane showed a mass 2 centimeters in diameter located immediately below the ulcerated epithelium. Histopathological examination showed a focal proliferation of large blood-filled vascular spaces lined by single layers of neoplastic elongated flattened cells, separated by thin connective tissue septae. The septae were infiltrated by lymphocyte aggregates (Figure 1). The

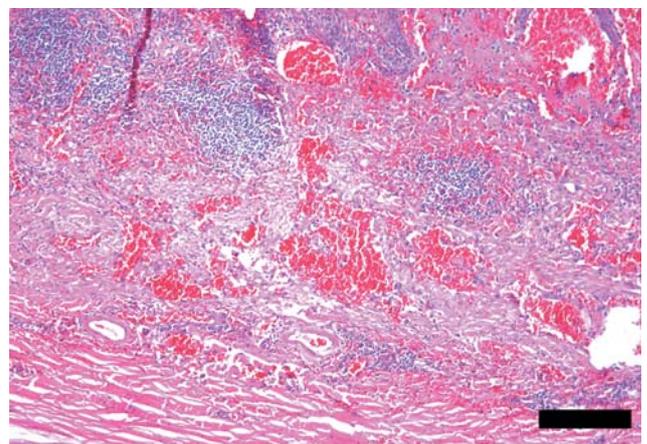


Figure 1. Section of the nictitans membrane mass stained with H&E showing proliferation of large blood-filled vascular spaces lined by single layers of endothelial cells, separated by thin connective tissue septae. The septae were infiltrated by lymphocyte aggregates.

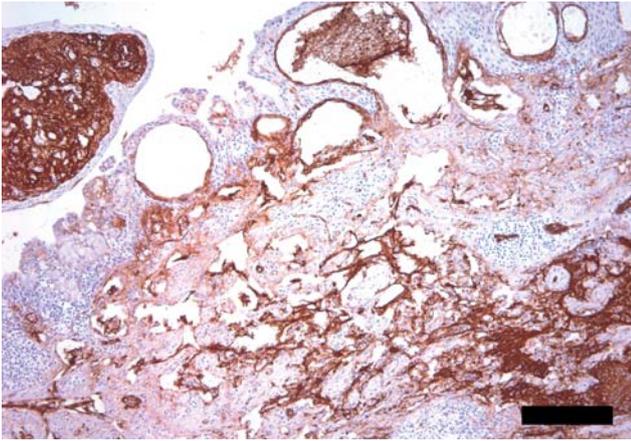


Figure 2. Section of the nictitans membrane mass showing a positive staining with von Willebrand Factor.

neoplastic cells showed irregular cytoplasmatic lining, rare mitotic figures and anisokaryosis. A 3-millimeter-thick margin of normal tissue surrounding the lesion was evident on microscopic evaluation. The neoplastic cells were identified as endothelial cells by the positive immunohistochemical staining for the von Willebrand Factor (Figure 2) (von Beust *et al.*, 1988). A cellular proliferation index of 8% was determined by Ki67 immunostaining (Figure 3). The tumor was diagnosed as a cavernous low-grade hemangiosarcoma because of the size of the vascular spaces and the amount of intervening fibrous tissue (Hargis and McElwain, 1984).

Six months after surgery, the horse had a complete ophthalmic recheck, and no recurrence of the neoplasm was found.

DISCUSSION

Hemangiosarcoma is an uncommon neoplasm in the horse. These tumors can occur in any organ that has a normal blood supply (Serenmoa *et al.*, 2000). Although rare in horses, they have been reported to occur in the subcutis (Hargis and McElwain; 1984), sinuses (Stencel and Groteleuschen; 1989), ovary and peritoneum (Waller and Rubarth; 1967), vagina (Tainturier and Wyers; 1987), pericardium (Birks and Hultgren; 1988), guttural pouches (Greene and O'Connor; 1986) and tarsal synovial sheath (Van Pelt *et al.* 1972). The equine third eyelid can also be involved, with a serosanguinous discharge being the most prominent symptom in 6 reported cases (Sansom *et al.*, 2006; Gearhart *et al.*, 2007). In the present case, a similar ocular discharge was seen. Hemangiosarcomas affecting the third eyelid have also been reported in cats (Multari *et al.*, 2002) and dogs (Liapis and Genovese; 2004).

The differentiation between hemangioma and hemangiosarcoma is extremely important in terms of prognosis. This is not always easy on the basis of clinical appearance or histopathology. Histological differentiation between hemangioma and low-grade hemangiosarcoma can be difficult if only a small biopsy is taken, especially in a standing horse prior to

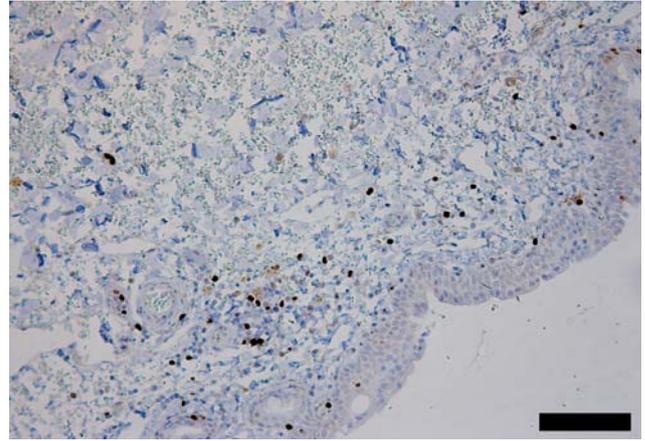


Figure 3. Section of the nictitans membrane with a Ki 67 immunostaining to determine the cellular proliferation index.

surgery (Sansom *et al.*, 2006). The results of the biopsy are used to guide surgical and treatment strategies (Gearhart *et al.*, 2007), so an exact diagnosis is important. In the present case, the nictitans membrane was almost completely removed and submitted for histological examination. Immunohistochemical staining for Factor VIII-related antigen was used to make the diagnosis of a blood vessel endothelial tumor. Ki67 allowed evaluation of the cellular proliferation. The Ki67 antigen is of major importance for the definitive diagnosis of hemangiosarcoma, since this antigen is a nuclear protein expressed in all of the active phases of the cell cycle (G_1 , G_2 , S, M), but not in resting cells (G_0). In a recent study involving 29 dogs, the proliferation index after Ki67 staining was less than 5% in hemangiomas (Sabattini and Bettini, 2008). No data on similar indexes have been reported in the literature for horses, but an index of 8% in combination with the histological observation of well demarcated vascular spaces lined by a single layer of cells strongly supports the classification as a low-grade malignancy.

The etiology of these vascular tumors remains unknown, although previous reports have proposed UV light as a risk factor (Hargis *et al.*, 1978). Since 1986, UV light has been recognized to be associated with skin cancer in humans. Several studies using laboratory animal models have demonstrated the carcinogenic effects of UV radiation (Kripke *et al.*, 1976). These findings suggest that the time spent outdoors is part of the etiology of conjunctival hemangiosarcomas in dogs (Pirie *et al.*, 2006). It has also been demonstrated that there is an increased risk of UV-induced neoplastic transformation in body areas lined by a thin epithelium, such as the conjunctival surface. Furthermore, the conjunctiva is naturally devoid of hair follicles and show a low degree of epithelial pigmentation. On the other hand, pigmentation is a significant inhibitory factor for the development of neoplastic skin processes (Kirby-Smith *et al.*, 1942). The horse in our case spent a large amount of time outdoors, but it is unknown whether solar effects had an influence on the development of the ocular tumor.

When cases are presented late in the course of the

disease, the tumor can have spread into the globe, orbit, sinuses and lymph nodes (Sansom *et al.*, 2006). Our patient was presented in a fairly early stage of the disease, since the lesion was only 2 centimeters in diameter and had not invaded the surrounding tissues. None of these tissues was further examined in the present horse, but it is most likely that no metastases had occurred since the horse showed no symptoms of relapse 6 months after the surgical intervention.

Hemangiosarcoma is a very aggressive tumor that may recur after excision and can metastasize even after aggressive surgical treatment (Hacker *et al.*, 1986). Therefore it has been classified as a neoplasm with an extremely poor prognosis. The time interval between finding the initial lesion and euthanasia has been reported in horses to be on average about 18 months (Hacker *et al.*, 1986). In contrast, the absence of recurrence and long-term survival after surgical excision of cutaneous hemangiosarcomas has been reported in other species (Frye *et al.*, 1983). In dogs, it has been suggested that cutaneous hemangiosarcomas are less likely to metastasize than tumors arising from internal sites (Johnson *et al.*, 1988). Although 6 months after the surgical intervention no significant problems or recurrence of the neoplasm had occurred in the present horse, a regular check-up for possible complications is still highly recommended.

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