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Equine conjunctival haemangiosarcoma: Clinical presentation, management, and outcome of seven cases in the United Kingdom

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ABSTRACT

Background: Only 27 cases of equine conjunctival haemangiosarcoma have been reported in the literature over the past 37 years. Out of these, 22% of cases were lost to follow-up, 52% were euthanized, and 26% survived. A scarcity of cases and information is available for this rarely seen conjunctival tumour.

Aim: To describe the clinical features, management, and outcome of conjunctival hemangiosarcoma in seven horses in the UK.

Methods: Optivet medical records were reviewed for equine cases seen or advised on with a histopathological diagnosis of conjunctival haemangiosarcoma between January 2013 and March 2023. Medical records were accessed for details of signalment, history, management, and follow-up. Histopathology was used to confirm the diagnosis of haemangiosarcoma and assess the surgical margins. Immunohistochemistry was performed in a minority of cases with poorly differentiated solid tumours to support vascular lineage.

Results: Seven eyes from seven horses (five geldings and two mares) with a mean age of 16 years and median of 18 years (range 10–21 years) met the criteria. Serosanguinous discharge was seen in six eyes. All eyes were managed surgically; 4 by exenteration and 3 by conjunctivectomy/keratectomy. Adjunctive cryotherapy was performed in two eyes. Metastatic disease in the ipsilateral parotid salivary gland, confirmed with histopathology, was seen in one horse. Surgical margins were clear in all but one eye. Solar elastosis was noted in five eyes. All horses were healthy at the last follow-up (0.2–5 years, mean 2.9 years, and median 2 years).

Conclusion: Equine conjunctival haemangiosarcoma is rare. Serosanguinous ocular discharge is a common clinical sign. Early surgical excision is highly effective. Solar elastosis is a common histopathological feature, suggesting a role for UV-light in the pathogenesis.

Keywords: Haemangiosarcoma, Solar elastosis, Horse, Vascular tumour, Ocular tumour.

Introduction

Primary tumours of vascular origin arise from the endothelium of blood or lymph vessels and can be benign or malignant. The equine eye is rarely affected, and both adnexal and intraocular forms have been reported (Hargis *et al*., 1978; Bolton *et al*., 1990). Primary conjunctival vascular tumours have been reported infrequently in several species including the cat (Multari *et al*., 2002; Pirie and Dubielzig, 2006), dog (Liapis and Genovese, 2004), horse (Vestre *et al*., 1982; Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006; Gearhart *et al*., 2007; Wegge *et al*., 2010; Pinn *et al*., 2011; Scherrer *et al*., 2018), cow (Sutton and McLennan, 1982; Ruggles *et al*., 1992), giant panda (Lopez *et al*., 1996), and humans (Honavar and Manjandavida, 2015). They originate from the endothelium of blood vessels within the conjunctiva associated with the limbus, the medial and lateral canthus, the third eyelid (TEL) and, rarely, from the cornea. The single report of primary corneal haemangiosarcoma postulated an origin from corneal neovascularization (Lavach and Severin, 1977; Vestre *et al*., 1982; Hacker *et al*., 1986; Crawley *et al*., 1987; Bolton *et al*., 1990). They are classified histologically as benign (haemangioma) or malignant (haemangiosarcoma) (Knottenbelt *et al*., 2015).

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Dogs are the most commonly affected species (Hargis *et al*., 1978; Pirie *et al*., 2006), and the lateral bulbar conjunctiva and the leading edge of the TEL are the most frequent locations (Hargis *et al*., 1978; Peiffer *et al*., 1978; Peiffer and Terrell, 1978; Pirie *et al*., 2006). Definitive diagnosis requires histological examination following biopsy (incisional or excisional), and the treatment of choice is surgical excision (Pirie *et al*., 2006). The prognosis in dogs is good; there is a risk of local recurrence, but metastatic disease has not been reported (Hargis *et al*., 1978; Peiffer *et al*., 1978; Peiffer and Terrell, 1978).

The most common ocular and adnexal tumour in horses is squamous cell carcinoma (SCC) (Lavach and Severin, 1977; Gearhart *et al*., 2007; Scherrer *et al*., 2018); it is also the second most common cutaneous tumour (Sundberg *et al*., 1977). Other tumours that present in the equine conjunctiva include papilloma, melanoma, mastocytoma, basal cell carcinoma, schwannoma, adenoma and adenocarcinoma, haemangioma, and haemangiosarcoma.

Older horses are predisposed (Sansom *et al*., 2006; Scherrer *et al*., 2018), and a serosanguinous ocular discharge is the main clinical feature (Sansom *et al*., 2006; Scherrer *et al*., 2018). Treatments have included surgical excision, enucleation, exenteration, surgical debulking with adjunctive therapies, radiation alone and euthanasia (Moore *et al*., 1986; Sansom *et al*., 2006; Scherrer *et al*., 2018). Adjunctive therapies included β-irradiation with strontium^{90}, iridium^{191}, brachytherapy, and cryotherapy (Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006).

Haemangiosarcoma is rare, with only 27 cases reported over almost four decades since 1986 (Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006, Gearhart *et al*., 2007; Wegge *et al*., 2010; Arenas-Gamboa and Mansell, 2011; Pinn *et al*., 2011; Scherrer *et al*., 2018). From these 27 cases, six were lost to follow-up (Arenas-Gamboa and Mansell, 2011), 14 were euthanized (Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006; Scherrer *et al*., 2018), and seven survived (Sansom *et al*., 2006; Wegge *et al*., 2010; Pinn *et al*., 2011; Scherrer *et al*., 2018). The seven surviving cases were all reported in more recent years (2006 onwards) when the aggressive nature of ocular and periocular haemangiosarcomas was becoming more apparent (Sansom *et al*., 2006; Wegge *et al*., 2010; Pinn *et al*., 2011; Scherrer *et al*., 2018) and treatment options were advancing.

Local recurrence and confirmed metastatic disease to the ipsilateral retropharyngeal lymph node has been reported in one horse (Gearhart *et al*., 2007); however, further follow-up was scarcely seen in the literature, the most commonly reported outcome was euthanasia without post-mortem (Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006; Gearhart *et al*., 2007; Pinn *et al*., 2011; Scherrer *et al*., 2018). Irrespective of the management of the cases, the poor outcome remained unchanged (Gearhart *et al*., 2007; Scherrer *et al*., 2018).

The aim of this study is to describe seven cases of equine conjunctival haemangiosarcoma in the UK in terms of clinical presentation, management, and outcome.

Materials and Methods

Study population

Optivet Referrals medical records were searched for equine cases seen or advised on with a histopathological diagnosis of conjunctival haemangiosarcoma between January 2013 and March 2023. Signalment, history, eye affected, duration of clinical signs, management, and follow-up were recorded. Phone calls to referring veterinary surgeons and owners were used to gather information pertaining to the current health status of the horse and its recurrence.

Histopathological evaluation and immunohistochemistry protocol

All tissue samples were fixed in 10% neutral buffered formalin and submitted for histopathological examination. Routine 4 µm hematoxylin and eosin (H&E)-stained sections were examined by a board-certified pathologist. Immunohistochemistry (IHC) was performed in poorly differentiated solid tumours to support vascular lineage as a requirement for further tumour identification. IHC was performed retrospectively in all cases where it had not been performed as part of the initial histopathological examination to investigate for concordance and to assess the value of immunohistochemical staining in the diagnosis of vascular tumours. Von Willebrand Factor (vWF, a vascular endothelial marker) was applied in all cases; vimentin (general mesenchymal marker) and cytokeratin (general epithelial marker) were applied in two cases.

The samples were initially pre-treated; this involved each sample being heated to 97°C, incubated for 20 minutes at 97°C and then cooled to 65°C. Following this, IHC was performed using the following specific antibodies:

– vWF—Polyclonal Rabbit: Anti-Human von Willebrand Factor. Dilution 1:6400
– Cytokeratin—Monoclonal Mouse : Anti-Human Cytokeratin clone—AE1/AE3
– Vimentin—Monoclonal Mouse : Anti-Vimentin clone—V9. Dilution 1:1000

IHC was performed using the Dako Autostainer Link 48 (Agilent, California, USA) using the streptavidin-biotin immunoperoxidase technique (Warren and Summers, 2007).

Ophthalmic examination

All horses underwent a full ophthalmic examination of both eyes, performed by a board-certified ophthalmologist or ophthalmology resident. Examination included slit lamp biomicroscopy (Kowa SL-15; Kowa; Japan), indirect ophthalmoscopy (Heine;

USA), applanation tonometry (Tonopen Vet; Reichert, USA) (after the application of topical anaesthesia with 0.5% proxymetacaine hydrochloride (Bausch + Lomb; Kingston, UK)), and fluorescein stain (Bausch + Lomb; Kingston, UK). Routine neuro-ophthalmic tests were assessed (palpebral reflex, menace response, dazzle reflex and direct/indirect pupillary light reflexes).

Sedation protocol

Surgical procedures were performed under standing sedation with intravenous detomidine at 30 mcg/kg (Domosedan, Vetoquinol, Towcester, UK) and butorphanol at 0.5 mg/kg (Torbugesic, Zoetis, Leatherhead, UK).

Nerve blocks

Surgery of the TEL was facilitated by local infiltration of 1–2 ml mepivacaine hydrochloride (Intra-Epicaine, Dechra, Northwich, UK) using a 25G 5/8" needle.

Akinesia and anaesthesia for enucleation and exenteration procedures were achieved with multiple local nerve blocks with mepivacaine hydrochloride (Intra-Epicaine, Dechra, Northwich, UK). Retrobulbar block, auriculopalpebral block, supraorbital nerve block, lacrimal nerve block, zygomatic nerve block and intratrochlear nerve blocks were performed in a routine manner. Topical anaesthesia was provided by 0.5% proxymetacaine hydrochloride (Bausch + Lomb; Kingston, UK) applied five minutes prior to surgery. If further desensitisation of the dorsomedial upper eyelid was required, local infiltration of mepivacaine hydrochloride (Intra-Epicaine, Dechra, Northwich, UK) was performed with a 25G 5/8" needle.

Surgical protocols

Biopsy

Incisional biopsy was performed, if required, before radical surgical procedures such as exenteration. An excisional biopsy was performed if the lesions appeared discrete and if excision was judged to be complete.

Exenteration

Standard exenteration included removal of the periosteum via a periosteal strip using periosteal elevators; the surgical site was closed with three layers of absorbable sutures. Intraorbital prosthetic implants were not used.

Keratectomy

Standard keratectomy and/or conjunctivectomy were performed in selected cases. The perimeter of the keratectomy site was demarcated with a 300 µm restricted depth blade (Sharpoint™ Wheel Knife, SJ Hales, Warwickshire, UK) to a depth of approximately 20%–40%, followed by lamellar dissection with a #64 Beaver blade, (SJ Hales, Warwickshire, UK). The surgical bed was not grafted.

Cryotherapy

Cryotherapy was performed either at the time of surgery or in the early post-operative period following the histopathological diagnosis. A compressed nitrous oxide cryotherapy unit (Cryomatic MKII Cryo Console, Keeler, Windsor, UK) was used. A closed probe was applied directly onto the surgical site, and a double freeze-thaw technique was used to achieve theoretical temperatures between -20°C and -40°C (Dugan *et al*., 1991).

Post-operative regimen

Post-operative medication comprised oral trimethoprim and sulfadiazine 30 mg/kg q12 hours (Trimediazine oral powder, Vetoquinol, Towcester, UK) and phenylbutazone 2.2 mg/kg q12 hours PO (Equipalazone, Dechra, Northwich, UK), both for five days. All horses had tetanus immunoprophylaxis. Topical medications varied by case.

Ethical approval

Ethical approval was not required for this study.

Results

Clinical presentation

There were five geldings and five mares; the ages ranged from 10 to 21 years, with a mean of 16.2 years (median 18 years) (Table 1). The duration of clinical signs prior to presentation ranged from 2 to 8 weeks, with a mean of 6.7 weeks (median 4 weeks). The left eye was affected in four cases and the right eye in three cases. Serosanguinous ocular discharge was the most common clinical sign, noted in six cases. Three out of seven cases arose from the conjunctiva of the TEL, and four out of seven cases arose from the bulbar conjunctiva. Three were from the lateral bulbar conjunctiva, and 1 was from the medial bulbar conjunctiva. From the four cases arising from the bulbar conjunctiva two cases had corneal involvement.

A difference in appearance was seen depending on location. The masses within the TEL all appeared contiguous with the conjunctiva and smoothly surfaced. The clinical appearance of the conjunctival masses on the TEL of cases 1, 3 and 7 are shown in Figure 1. The masses on the bulbar conjunctiva appear to be exophytic, lobulated and discrete. The clinical appearance of the bulbar conjunctival masses of cases 2, 5 and 6 are shown in Figure 2.

Management

All cases were treated with surgical excision: incisional biopsy (n = 2), excisional biopsy (n = 3), and no biopsy prior to exenteration (n = 2). Five exenterations were performed, 3 following a prior biopsy and 2 without biopsy (Table 2). Excisional biopsy included combined conjunctivectomy/keratectomy, (n = 2) and TEL excision (n = 1). Six cases had clear margins with the initial surgery. Unclear margins were not achieved in a case (case 4 with combined lamellar keratectomy-conjunctivectomy). Despite subsequent exenteration 4 weeks later, metastatic spread to the ipsilateral parotid salivary gland was confirmed histopathologically. Adjunctive cryotherapy was performed in two cases that had combined conjunctivectomy and keratectomy; case 5 had cryotherapy at the time of surgery, and case 6 had cryotherapy performed 2 months post-operatively; neither of these cases showed recurrence. Investigation

Table 1. Signalment, clinical signs, and description of the masses.

	Signalment	Clinical signs	Description of mass
Case 1	- 21-year old - Grey - Thoroughbred - Gelding	- Serosanguinous ocular discharge - Prominence of TEL	- Hyperaemic mass contiguous with TEL - Gross dimensions could not be obtained
Case 2	- 18-year old - Palomino - Arab-cross - Gelding	- Serosanguinous ocular discharge - Partial lagophthalmos	- Exophytic hyperaemic mass - 12 × 6 × 4 mm
Case 3	- 18-year old - Grey - Cob - Gelding	- Serosanguinous ocular discharge - Lower eyelid displacement caused by TEL mass	- Exophytic hyperaemic mass - 13 × 10 × 3 mm
Case 4	- 18-year old - Grey - Welsh Section A - Gelding	- Red mass on eye - Rapid increase in size 2 weeks prior to referral	- Slightly exophytic hyperaemic mass - 14 × 9 × 2 mm
Case 5	- 10-year old - Grey - Connemara - Mare	- Serosanguinous ocular discharge - Red mass on eye	- Exophytic hyperaemic lobulated mass - 9 × 8 × 6 mm
Case 6	- 18-year old - Piebald - American Paint - Gelding	- Serosanguinous ocular discharge - Red mass on eye	- Exophytic multilobulated hyperaemic mass - 4 × 3 × 2 and 2 mm from the limbus
Case 7	- 11-year old - Grey - Irish draught-cross - Mare	- Serosanguinous ocular discharge - Prominence of TEL	- Exophytic hyperaemic mass - 15 × 14 × 3 mm

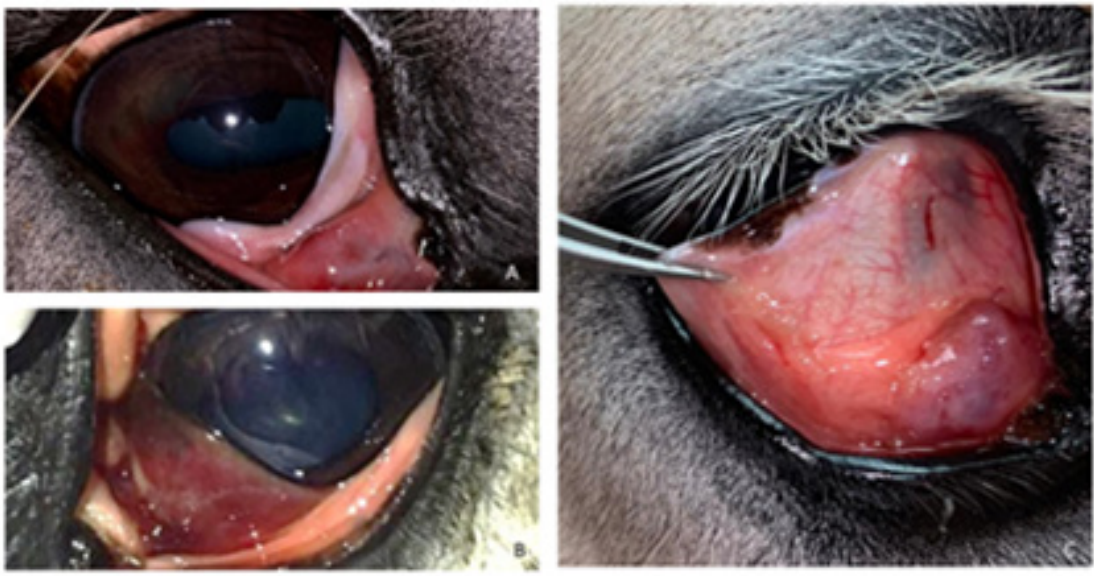

Fig. 1. Third eyelid haemangiosarcoma/angiosarcoma gross presentation. (A) Case 3 conjunctival angiosarcoma, (B) Case 1 conjunctival haemangiosarcoma, (C) Case 7 conjunctival haemangiosarcoma. Hyperaemic appearance and contiguous nature of the tumour are evident in all three cases.

Fig. 2. Bulbar conjunctival haemangiosarcoma gross presentation. (A) Case 2 conjunctival and corneolimbal haemangiosarcoma, (B) Case 6 bulbar conjunctival haemangiosarcoma, (C) Case 5 bulbar conjunctival haemangiosarcoma. Note the exophytic, discrete and lobulated appearance of the tumour.

Table 2. Location, surgical procedure performed, and outcome.

	Mass location	Surgery performed	Outcome
Case 1	- Bulbar conjunctival TEL	- Incisional biopsy - Exenteration after histopathological confirmation of HSA	Alive at 5 years
Case 2	- Lateral bulbar conjunctiva	- No biopsy - Exenteration	Alive at 5 years
Case 3	- Anterior palpebral conjunctiva TEL	- No biopsy - Exenteration	Alive at 18 months
Case 4	- Dorso-medial bulbar conjunctiva	- Incisional biopsy - Exenteration 4 weeks post biopsy	Metastasis to ipsilateral parotid salivary gland 9 months post-operatively. Alive at 4 years
Case 5	- Lateral bulbar conjunctiva	- Excisional biopsy - Cryotherapy at time of surgery	Alive at 2 years
Case 6	- Dorso-lateral bulbar conjunctiva	- Excisional biopsy - Cryotherapy performed 2 months post-surgery	Alive at 14 months
Case 7	- Anterior palpebral conjunctiva TEL	- Excisional biopsy - Exenteration after histopathological confirmation of HSA	Alive at 2 months

into metastasis was performed in three cases, and no abnormalities indicative of metastasis were reported. Case 4 with metastatic spread did not have further imaging performed.

Histopathology

Five eyes from five horses were diagnosed with conjunctival haemangiosarcoma, and two eyes from two horses were diagnosed with conjunctival angiosarcoma with a high suspicion of haemangiosarcoma (Table 3).

In the five horses diagnosed with conjunctival haemangiosarcoma, the histopathology findings confirmed the presence of a clearly vasoformative neoplasm in each case with the neoplastic endothelial cells either lining blood-filled vascular channels and/or forming solid nests (Fig. 3). Multiple lymphonodular aggregates or lymphoid follicles were commonly interspersed throughout the neoplastic infiltrate.

Table 3. Histopathological findings and IHC results.

	Solar elastosis	Histopathological diagnosis	IHC performed
Case 1	yes	TEL conjunctival haemangiosarcoma	Yes—retrospectively vWF—10% positive
Case 2	yes	Conjunctival and corneolimbal haemangiosarcoma	Yes—retrospectively vWF—20%–30% positive
Case 3	yes	TEL conjunctival angiosarcoma (high suspicion of haemangiosarcoma)	Yes—at the time vWF—<1% positive Vimentin—>95% positive Cytokeratin—negative
Case 4	no	Conjunctival angiosarcoma (high suspicion of haemangiosarcoma)	Yes—at the time vWF—80%–90% positive Vimentin—>95% positive cytokeratin—negative
Case 5	yes	Conjunctival haemangiosarcoma	Yes—retrospectively vWF—5%-10% positive
Case 6	yes	Conjunctival haemangiosarcoma	Yes—retrospectively vWF—30% positive
Case 7	no	TEL conjunctival haemangiosarcoma	Yes—retrospectively vWF—negative

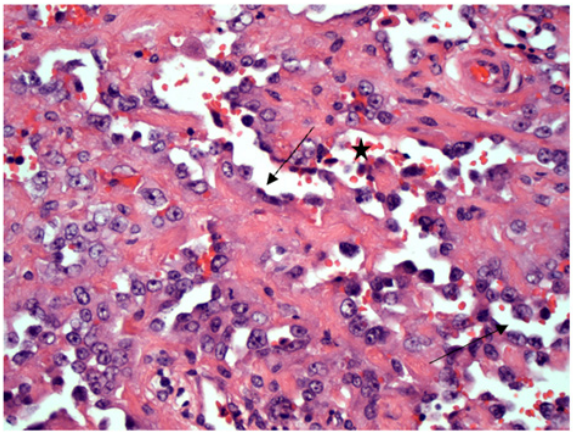

Fig. 3. H&E photomicrograph (400× magnification) of case 1 conjunctival haemangiosarcoma from the third eyelid of a horse. Irregular blood-filled vascular channels (star) formed by the tumour and lined by pleomorphic neoplastic endothelial cells (arrows).

Moderate to marked cellular pleomorphism and nuclear atypia were seen in all cases. Mitoses per 10HPF (2.73 mm^2), varied from 3 to 34 per HPF, with case 4 displaying the highest number of mitoses and the only case with metastatic spread (Fig. 4). Solar elastosis was seen in five cases (Fig. 5).

In the two horses diagnosed with conjunctival angiosarcoma, the histopathological findings reflected malignant conjunctival neoplasms that were poorly differentiated with regards to lineage. Both of these neoplasms had a solid epithelioid growth pattern and an absence of vascular channel formation,

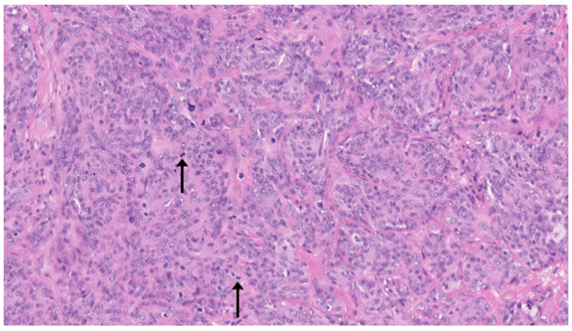

Fig. 4. H&E photomicrograph (20× magnification) of case 4, an angiosarcoma from the bulbar conjunctiva of a pony. Solid nests of neoplastic epithelioid cells devoid of vascular channel formation. Frequent mitoses are evident (arrows).

but vascular lineage was suspected given the location and H&E morphology of the tumour, accompanying perivascular lymphonodular aggregates and absence of overlying surface epithelial pathology with regards to dysplastic or neoplastic changes to indicate carcinoma (Fig. 6). IHC was performed in these two cases in an attempt to rule out the differential diagnosis of carcinoma and confirm vascular lineage as a requirement for further tumour identification (Fig. 7).

IHC was performed retrospectively in all cases to investigate for concordance and to assess the value of immunohistochemical staining. Von Willebrand Factor (vWF, a vascular endothelial marker) was applied in all cases; vimentin (generalised mesenchymal marker) and cytokeratin (epithelial marker) were applied in the two poorly differentiated tumours at the time of diagnosis.

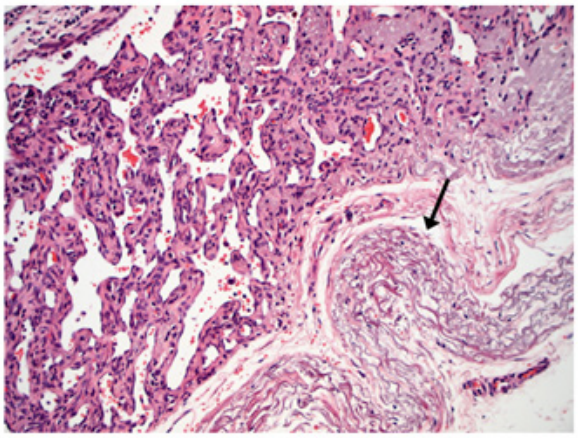

Fig. 5. H&E photomicrograph (100× magnification) of case 2 bulbar conjunctival haemangiosarcoma from a horse characterised by irregularly branching, blood-filled vascular channels. There is evidence of solar elastosis in the conjunctival stroma (arrow).

Follow-up and outcome

All horses were in good health at the last follow-up, which ranged from 2 months up to 5 years, with a mean of 2.9 years (median 2.5 years).

Statistical Analysis

Due to categorical data and small sample sizes, a Fisher's exact test was performed with significance set at 95% confidence level (p-value <0.05). Contingency tables were created by comparing pairwise the survival cases number from this work against the survival cases of each study where conjunctival haemangiosarcoma was diagnosed (Table 4) (Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006; Gearhart *et al*., 2007; Wegge *et al*., 2010; Pinn *et al*., 2011; Scherrer *et al*., 2018).

Studies by Wegge and Pinn were excluded from individual calculations due to the sample size consisting of just one case. These were included when comparing our findings to the broader literature (Table 5).

Discussion

Survival

This study reports a series of five cases of equine ocular haemangiosarcoma and two cases of equine ocular angiosarcoma (with a high suspicion of haemangiosarcoma). All cases were treated surgically, with two cases receiving adjunctive cryotherapy, and had a 100% survival rate at a mean of 2.9 years (34.8 months). This is in contrast to previous studies that demonstrated a high rate of euthanasia rate (73.6%) (Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006; Gearhart *et al*., 2007; Scherrer *et al*., 2018).

Age

Conjunctival haemangiosarcoma appears to be a disease of older horses with an age range in the literature seen from 8 to 25 years old and the average age is 15.2 years (Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006; Gearhart *et al*., 2007; Arenas-Gamboa and Mansell, 2011; Pinn *et al*., 2011; Scherrer *et al*., 2018). In this study, five out of seven horses were categorised as geriatric (aged 15 years or over) (McGowan, 2011). The age range of the horses in the current study was 10–21 years and an average age of 16.2 years. This is consistent with the previous literature (Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006; Gearhart *et al*., 2007; Wegge *et al*., 2010; Pinn *et al*., 2011; Scherrer

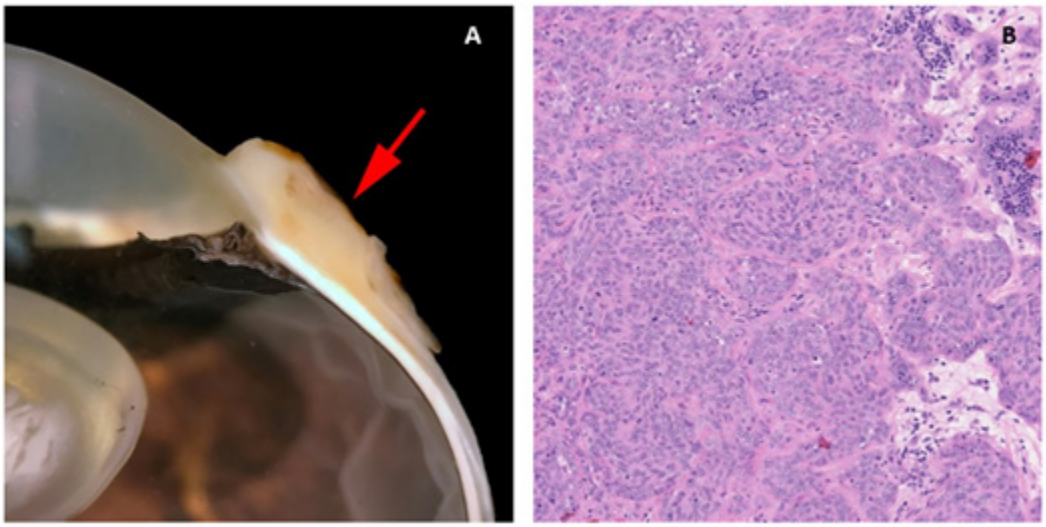

Fig. 6. (A) Case 4 gross pathology photomicrograph of a solid, poorly differentiated angiosarcoma, note the cream-coloured cut surface. (B) Histopathology photomicrograph, case 4, note the highly cellular and solid presentation with a lack of vasculature and red blood cells.

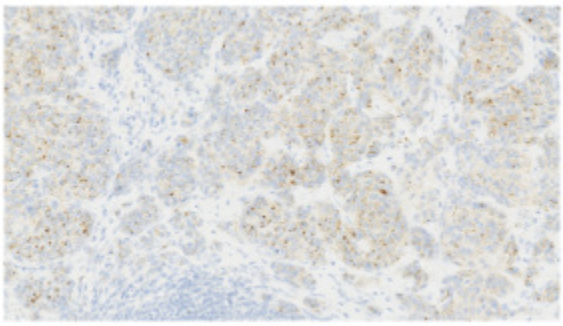

Fig. 7. *vWF stained photomicrograph (×20 magnification) of case 4 angiosarcoma from the bulbar conjunctiva of a pony. The majority of neoplastic cells are immunopositive for vWF (IHC, 3,3'-Diaminobenzidine DAB chromogen). Confirming an endothelial cell origin to the tumour.*

et al., 2018). The findings of this study, along with the literature, support that conjunctival haemangiosarcoma is a disease of older horses.

Clinical signs

The most common clinical sign of equine conjunctival haemangiosarcoma in this case series was a serosanguinous ocular discharge, which was present in six of the seven horses (85.7%). This is in accordance with previous reports (75%) (Sansom *et al*., 2006; Scherrer *et al*., 2018). A serosanguinous ocular discharge is not seen with haemangioma, SCC or other tumour types at this location, and so its presence, particularly when of significant duration and refractive to first-line treatment, should raise the clinical index of suspicion for haemangiosarcoma and prompt further investigation (Giuliano, 2010; Malalana, 2022).

Predisposition

All seven horses had a pale or dilute coat colour, which has been shown to be a predisposing factor in other studies (Sansom *et al*., 2006; Gearhart *et al*., 2007; Pinn *et al*., 2011; Scherrer *et al*., 2018). Previous literature also highlighted increased ultraviolet light exposure and being kept outside as potential risk factors (Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006; Gearhart; *et al*., 2007; Wegge *et al*., 2010; Pinn *et al*., 2011).

Six of the seven horses had histopathological features of solar elastosis. In a canine population, 39 of 46 dogs with cutaneous haemangiosarcoma had solar elastosis in the skin adjacent to the tumour (Hargis *et al*., 1978). The presence of solar elastosis is a risk factor for SCC in horse (Campbell *et al*., 1987) but has not been reported for cases of haemangiosarcomas. Tumour location is often thought to be associated with areas of greatest ultraviolet (UV) light exposure (e.g., the leading edge of TEL and temporal bulbar conjunctiva), suggesting a role for UV light; however, the exact pathogenesis warrants further research.

Location and prognosis

The biological behaviour and prognosis associated with haemangiosarcomas are based on its location and the species affected. (Sorenmo *et al*., 2000; Schultheiss, 2004). Non-visceral ocular and peri-ocular locations are relatively uncommon locations for this tumour type in all species, with dogs being most frequently affected, followed by cats and then horses (Richardson and Deykin, 2021). A retrospective study investigating canine and feline non-visceral haemangiosarcomas identified excision with clear margins as a successful treatment with favourable outcomes in these species (Schultheiss, 2004). Previous studies have postulated that haemangiosarcomas do not display the same behaviour in the ocular and peri-ocular locations as in the cutaneous, visceral or disseminated forms (Scherrer *et al*., 2018). The available results from this study and others suggest that a positive outcome in horses is likely because of the possibility of obtaining clear surgical margins (Scherrer *et al*., 2018).

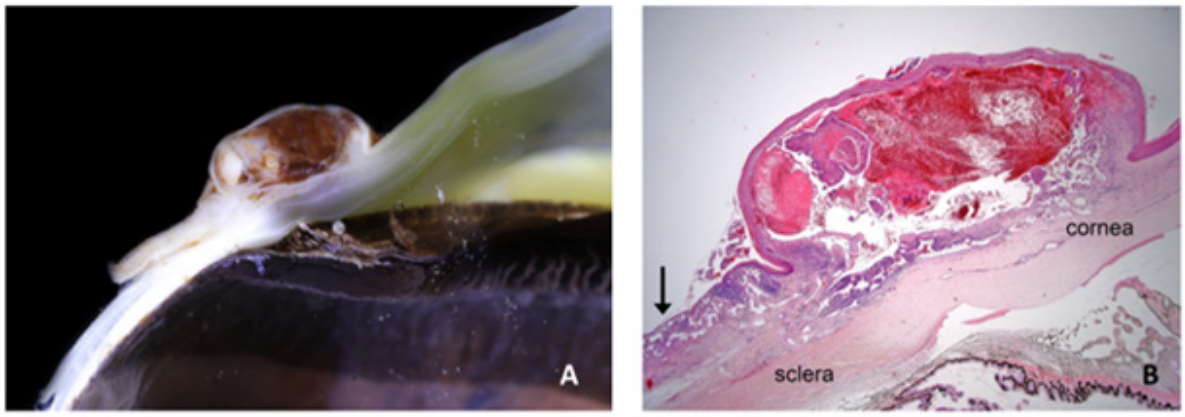

Fig. 8. *(A) Case 2 gross pathology photomicrograph revealing the lobulated presentation and red colour to the cut surface. (B) Case 2 low-power histopathology photomicrograph revealing the well-differentiated haemangiosarcoma with blood-filled vessels, note the invasive nature of the tumour tissue (black arrow).*

Table 4. Comparison of survival of horses in this study versus survival of horses in other studies in the literature.

Author	Total number of horses	Number of horses "not survived"	Number of horses "survived"
Our study	7	0	7
Scherrer *et al.* (2018)	6	2	4
Sansom *et al.* (2006)	4	3	1
Gearhart *et al.* (2007)	1	1	0
Hacker *et al.* (1986)	2	2	0
Pinn *et al.* (2011)	1	0	1
Moore *et al.* (1986)	4	4	0
Bolton et al. (1990)	2	2	0
Wegge *et al.* (2010)	1	0	1
Total	**28**	**14**	**14**

Table 5. Contingency table assessing pairwise survival outcomes from this study to each individual study in the literature and calculated *p*-values.

Contingency test	Number of horses "not survived"	Number of horses "survived"	*p*-value
Scherrer *et al.* (2018)	2	4	0.19230
Our study	0	7	
Sansom *et al.* (2006)	3	1	0.02424
Our study	0	7	
Hacker *et al.* (1986)	2	0	0.0278
Our study	0	7	
Moore *et al.* (1986)	4	0	0.003
Our study	0	7	
Bolton et al. (1990)	2	0	0.0278
Our study	0	7	
All papers	14	14	0.0272
Our study	0	7	
All papers (excluding this study)	14	7	0.0058
This paper	0	7	

Surgery

Lesions were excised with clear margins in six of the seven horses (85.7%). There was a recurrence in the one horse that did not have clear margins at the initial surgery. Although clear margins were subsequently obtained with exenteration 4 weeks later, metastatic spread to the ipsilateral parotid salivary gland was later confirmed. This supports the findings in a recent case series of ocular and peri-ocular haemangiosarcoma—complete excision at the initial surgery resulted in no recurrence in four of six horses (Scherrer *et al*., 2018). The two cases with post-excisional recurrence had histologic evidence of incomplete excision (Scherrer *et al*., 2018). The treatment options include surgical excision (conjunctivectomy, keratectomy, nictitating membrane removal, enucleation, and exenteration) with or without adjunctive therapies (cryotherapy or radiation therapy), or euthanasia (Hacker *et al*., 1986; Moore *et al*., 1986; Bolton *et al*., 1990; Sansom *et al*., 2006; Gearhart *et al*., 2007; Scherrer *et al*., 2018).

There are only three reports of a successful outcome for equine ocular and periocular hemangiosarcoma (Wegge *et al*., 2010; Pinn *et al*., 2011; Scherrer *et al*., 2018). Survival was greater than 18 months in those three cases, two involving the TEL and one conjunctival mass (Wegge *et al*., 2010; Pinn *et al*., 2011; Scherrer *et al*., 2018). These cases, combined with those from our study, reveal that 8 out of 11 horses had a successful outcome when complete excision with clear margins was performed at the initial surgery.

Metastatic disease in the ipsilateral parotid salivary gland was identified in one horse (case 4) (Kan *et al*., 2011). The clinical signs of facial nerve damage seen in this case were attributed to the parotid gland biopsy due to the course of the facial nerve over the parotid gland and their spontaneous resolution. (Boorman *et al*., 2020; Zetterström *et al*., 2022). The parotid gland swelling could be attributed to neoplastic infiltration and inflammation. The spontaneous resolution of the swelling without surgical excision, chemotherapy or radiotherapy may have been due to the non-steroidal anti-inflammatory treatment given after the surgical biopsy. Alternatively, or as well as this, an innate immune response (such as that mediated by natural killer cells) could have destroyed any small population of neoplastic cells present.

Statistical significance

Through the analysis of individual study results, we gained valuable insights into potential associations between survival and specific factors examined in this study, including age, dilute coat colour, solar elastosis, and serosanguinous ocular discharge. Comparing these findings with the available literature, we observed that the null hypothesis was not discarded only in the comparison between samples from this study (Scherrer *et al*., 2018). Remarkably, the seven survival cases from our work exhibited *p*-values below 0.05 in all other individual comparisons, strongly rejecting the null hypothesis. Furthermore, when compared with the total number of survival cases found in the literature, this rejection becomes even more pronounced, suggesting that the presence of seven survival cases is not merely a chance occurrence but likely due to an underlying contributing factor in this study.

Given the limited number of samples available, it is challenging to determine the specific contributing factors to the survivability of our cases. The most suitable approach for addressing this question would involve Bayesian modelling. However, conducting such an analysis requires careful consideration of prior distributions and a more in-depth investigation of the relevant factors. Regrettably, this level of complexity is beyond the scope of the present work. Nevertheless, it opens the possibility for future research to build upon our findings and explore the factors influencing survivability in a more comprehensive and sophisticated Bayesian framework.

Histopathology

Angiosarcoma is an umbrella term for tumours that are classified as malignant masses that originate within the vascular system (Fosmire *et al*., 2004) and can be further classified into haemangiosarcoma (tumours originating from the vasculature of blood vessels) or lymphangiosarcoma (tumours originating from the vasculature of the lymphatic system) (Halsey *et al*., 2016). Microscopically, the cell morphology of both tumour types is similar (Kim *et al*., 2015), and if the endothelial cell origin/lineage is unable to be categorised to the lymphatic system or the vascular system, then immunohistochemical markers can be employed (Fosmire *et al*., 2004). A diagnosis of haemangiosarcoma is made by the presence of erythrocytes within vascular channels formed by the tumour; lymphangiosarcoma is diagnosed by the absence of erythrocytes within the vascular channels (Swayne *et al*., 1989; Diessler *et al*., 2003; Halsey *et al*., 2016). The cases included in this study were all confirmed as haemangiosarcomas histopathologically or were deemed angiosarcoma with a high suspicion of haemangiosarcoma.

Definitive diagnosis, therefore, always requires histopathological analysis of a biopsy sample (incisional or excisional), and so it is an important consideration for clinicians who are considering surgical intervention for ocular or periocular lesions.

In Figure 8, a conjunctival haemangiosarcoma displays the more "classical" gross appearance of haemangiosarcoma; an exophytic mass with red appearance to both the cut surface and on histopathology. Blood-filled channels lined with neoplastic cells provide the tumour with a red appearance. Although clinically appearing as a discrete mass, the invasive nature of this tumour type can be seen with neoplastic cells infiltrating the cornea outside the mass. Contrast this to Figure (6), a poorly differentiated, solid angiosarcoma. Whilst still marginally exophytic, the gross appearance was cream/pale coloured, and histopathology revealed a lack of blood vessels. The tumour was solid in appearance with poorly differentiated cells making classification difficult. This highlights the importance of haemangiosarcoma as a differential for conjunctival masses in a horse, even if their appearance is not grossly red.

IHC is not always required for a diagnosis, and in this case series, it was only indicated in two of the seven horses. The remaining five cases had histopathologic findings typical of haemangiosarcoma, and examination of H&E sections alone was sufficient for the diagnosis. IHC is typically performed on a poorly differentiated tumour to help further categorise it when defining morphological features of cell lineage are absent. Vimentin, cytokeratin and vWF immunohistochemical markers were performed in the two cases (Cases 3 and 4) where a solid conjunctival epithelioid neoplasm was diagnosed: vascular lineage was suspected, but IHC was

performed in an attempt to confirm this and rule out the less likely differential diagnosis of carcinoma. Vimentin is a structural protein, the presence of which confirms a mesenchymal origin for a given tumour (Ifedioranma *et al*., 2020) but is otherwise not specific. Cytokeratin is a protein that provides mechanical support to epithelial cells and, therefore, confirms an epithelial cell origin for tumours. Both cases (Case 3 and 4) in this case series were immunonegative for cytokeratin and, therefore, did not support the differential diagnosis of carcinoma. vWF (also known as factor VIII-related antigen) is a vascular endothelial marker, confirming the origin of tissue to endothelial cells of the vascular system, e.g., an angiosarcoma (Smith *et al*., 1989). In one of the cases (Case 4), the majority of neoplastic cells were strongly immunopositive for vWF, and in the second case (Case 3), only rare neoplastic cells were immunopositive for vWF. The diagnosis of conjunctival angiosarcoma was made in both cases based on the location of the tumour, the H&E findings and the combination of immunochemistry results as outlined above. It is not uncommon for IHC to show a variability in the strength of immunostaining and/or the distribution pattern of immunostaining including the percentage of immunopositive neoplastic cells, particularly in the case of poorly differentiated malignancies. It is important to realise that although an immunopositive result can be helpful, an immunonegative result does not necessarily exclude the cell lineage being tested for depending on the level of sensitivity of the antibody for that particular neoplastic cell lineage in that particular species.

Interestingly, vWF IHC was retrospectively performed in all remaining five cases of haemangiosarcoma and revealed a wide variation in immunostaining strength and pattern. Findings were measured against a consistent positive internal control (endothelial cells lining normal blood vessels). Up to a maximum of 30% neoplastic cells were immunopositive for vWF), in five cases (Cases 1, 2, 3, 5, and 6), 80%–90% neoplastic cells were immunopositive in one case (Case 4), and neoplastic endothelial cells were immunonegative in one case (Case 7) despite a clear histopathological diagnosis of haemangiosarcoma. This suggests that this particular antibody with the specific methodology and antibody dilution used in this case series of horses was not highly sensitive for labelling neoplastic endothelial cells and that vascular lineage could not be reliably excluded in a poorly differentiated neoplasm suspected to be an angiosarcoma if the neoplastic cells were immunonegative for vWF.

This study highlighted the fact that their use does not always support the diagnosis fully and should only be utilised in cases of poorly differentiated tumours where a definitive diagnosis cannot be achieved with histopathological analysis alone. They can help to provide supplementary supporting information in an equivocal case but should not be relied on solely for the diagnosis of a haemangiosarcoma.

There are no immunohistochemical stains routinely available that differentiate haemangiosarcoma from lymphangiosarcoma definitively. Two novel lymphatic endothelial cell-specific (LECs) markers, LYVE-1 and PROX-1, have been identified (Halsey *et al*., 2016) and are, in specific laboratories, available for the further differentiation of the two tumour types. These markers have yet to be validated in horses.

Study limitations

The retrospective nature of the study has limitations, including consistency with the diagnostic approach, management, and medical therapy. The number of cases is low, which reflects the condition being uncommon; case 7 was included despite only two months follow-up due to the uncommon presentation of this disease. Incisional biopsies were not performed prior to exenteration in two cases because of financial constraints; the authors recommend that a biopsy (incisional or excisional) should always be performed prior to enucleation or exenteration.

Conclusion

Equine ocular and periocular haemangiosarcomas are infrequently seen tumours; they appear to occur in older horses with pale coat colour. A persisting serosanguinous ocular discharge is a common clinical sign. A successful outcome can be achieved with early surgical intervention and complete excision at the initial surgery. The histopathological confirmation of clear surgical margins is important for the prognosis. Ultraviolet light may play a significant role in the aetiopathogenesis of ocular haemangiosarcoma.

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Authors contributions

Andrea Kashani-Carver: Main author and contributor. Performed clinical examinations of some cases, performed owner phone calls, and drafted the full manuscript, excluding the statistical analysis section. Conor O'Halloran: Helped to edit and review the manuscript. Emma Scurrell: Prepared and interpreted all samples, reviewed all histopathology, generated images for the manuscript, and provided critical review. Heidi Featherstone: Helped to critically review and edit multiple manuscript drafts and provide supervision. Felipe Ferreira de Freitas: Data scientist. The drafted manuscript section on statistical analysis and provided tables. Rob Lowe: Supervisor of main author and paper.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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