CASE REPORT

Retinoblastoma in the eye of a llama (*Llama glama*)

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Abstract

Animal studied A 6-year-old, pregnant female llama experienced a 6-month history of epiphora, buphthalmos, and acute loss of vision in the left eye. The condition was unresponsive to topical antimicrobial and anti-inflammatory therapy and progressed to corneal rupture.

Procedures Transpalpebral enucleation was performed and an intraorbital silicone prosthesis was implanted. The eye was fixed in formalin and processed according to routine paraffin technique. Sections of a mass were immunohistochemically prepared routinely and stained for glial fibrillary acidic protein (GFAP), S-antigen, and rhodopsin.

Results Gross, histopathologic, and immunohistochemical analysis revealed a retinal tumor consistent with a retinoblastoma. The neoplastic tissue formed Flexner–Wintersteiner and Homer–Wright rosettes, originated from the retina, and demonstrated photoreceptor differentiation with S-antigen and rhodopsin expression. Neoplastic cells were negative for GFAP. Four years after enucleation, the llama showed no signs of recurrent neoplasia.

Conclusions This report describes the diagnosis and successful treatment of the first known retinoblastoma in a llama.

Key Words: enucleation, llama (*Llama glama*), retinoblastoma, rhodopsin, rosettes, S-antigen

INTRODUCTION

Retinoblastomas are the most common primary intraocular malignancy in children. The hereditary form of retinoblastoma in humans has been associated with a mutation in the tumor suppressor gene (*rb*) located on human chromosome 13q14.[1–4] Mutation of this gene predisposes affected children to intraocular retinoblastomas as well as intracranial neoplasia.[5–7] There remains considerable debate regarding whether retinoblastomas should be diagnosed in animals without confirmation of the *rb* mutation.[1,2,8]

Ocular neoplasms morphologically resembling retinoblastomas have been diagnosed as medulloepitheliomas and reported in two llamas, and several other animal species including dogs, cats, horses, chickens and cockatiels.[1,2,8–18] Medulloepitheliomas are derived from the neuroepithelium of the optic cup or vesicle and have the potential to develop into retinal pigment epithelium, ciliary epithelium, neural epithelium, neuroglia, or vitreous.[11]

Although intraocular neoplasms are rare in llamas, neoplasia should be considered as a differential diagnosis for ocular disease in this species.[17,18] The present case describes the successful treatment of a unilateral retinoblastoma in the eye of an adult llama.

CASE REPORT

A 6-year-old, pregnant female llama was referred to Purdue University Veterinary Teaching Hospital with a 6-month history of epiphora, buphthalmos, and acute loss of vision in the left eye. The eye had been treated with topical antibiotics and an oral, nonsteroidal, anti-inflammatory drug (acetylsalicylic acid). Despite this therapy the eye continued to enlarge and the cornea became diffusely edematous, extensively ulcerated, and eventually ruptured prior to referral.

At presentation, the llama had normal vital parameters and exhibited epiphora and blepharospasm of the left eye. The left eye had a 1-cm diameter, well-circumscribed, full-thickness

corneal ulcer that exuded purulent discharge. The area of corneal rupture was partially covered by a pseudomembrane and was surrounded by granulation tissue. The cornea was diffusely edematous with peripheral neovascularization. Corneal opacity precluded examination of the lens and fundus. No abnormalities were detected in the right eye. A viable fetus of approximately 10 months gestation was detected by rectal examination and transabdominal ultrasonography.

MATERIALS AND METHODS

Enucleation of the affected eye was recommended to the owners. Preoperatively, the llama was administered ceftiofur (2.2 mg/kg [1 mg/lb] IV, q12 h; Pfizer Animal Health, New York, NY, USA) and flunixin meglumine (1 mg/kg [0.45 mg/lb] IV, q12 h; Schering Plough Animal Health, Kenilworth, NJ, USA) and placed under general anesthesia. Transpalpebral enucleation of the left eye was performed as previously described.[19] An encircling ligature was applied to the optic nerve with 0 polydioxanone. For cosmetic purposes a silicone prosthesis, 43 mm in diameter (Jardon Implants, Southfield, MI, USA), was implanted into the orbit. The prosthesis was maintained within the orbit by three simple interrupted sutures of 0 polydioxanone attached to the dorsal and ventral periorbital fascia evenly spaced along the orbit. Deep and superficial subcutaneous layers were apposed individually with 2-0 polyglactin 910. The skin was closed with 2-0 polydioxanone in a simple interrupted pattern. The llama was continued on flunixin meglumine for 24 h, ceftiofur for 5 days and then discharged.

The vitreous chamber of the enucleated eye was infused with approximately 1 mL of 10% buffered formalin and then immersed in formalin for 1 week. The eye was then sequentially immersed in a series of ethanol solutions, beginning with 70% and ending with 95%, over a 3-week period. Once firmly fixed the eye was sectioned in the sagittal plane for gross and histologic evaluation.

Immunohistochemistry was performed on sections of neoplastic tissue using routine protocols.[20] Briefly, sections of neoplastic tissue were deparaffinized and rehydrated by routine methods. Antigen retrieval was accomplished by incubation of slides in antigen retrieval solution (Dako Cytomation, Carpinteria, CA, USA) in a steamer (Black & Decker® US Inc., Towson, MD, USA) for 20 min. Endogenous peroxidase was blocked for 15 min with 3% hydrogen peroxide. Nonspecific immunoglobulin binding was blocked by incubation of slides for 10 min with a protein-blocking agent (Dako Cytomation) prior to application of the primary antibody. The latter was allowed to react for 30 min at room temperature. Sections were stained in a Dako autostainer apparatus. The slides were incubated with antibodies against glial fibrillary acidic protein or GFAP (1 : 1600) (Dako Cytomation), S-antigen (1 : 400) (gift from Dr PA Hargrave, University of Florida), and rhodopsin (1 : 1000) (Laboratory Vision Corporation, Fremont, CA, USA). A streptavidin-immunoperoxidase staining procedure (Dako Cytomation) was used for immunolabeling. The immunoreaction was 'visualized' with 3,3′-diaminobenzidine substrate (Dako Cytomation). Sections were counterstained with Mayer's hematoxylin. Positive immunohistochemical controls included a normal eyeball from a llama to which the appropriate antisera were added. For negative controls the primary antibodies were replaced with homologous nonimmune sera.
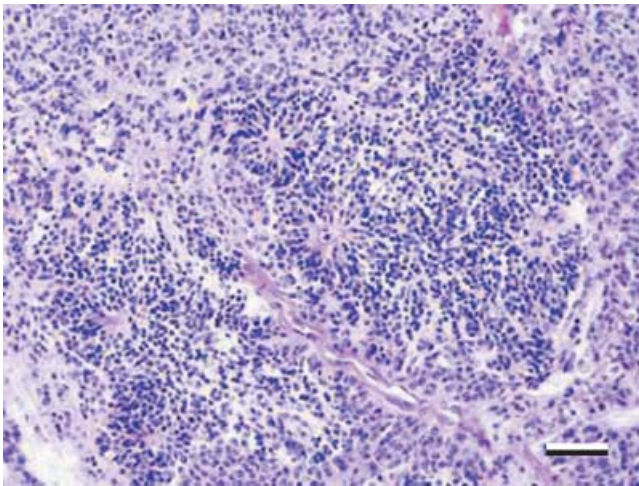
RESULTS

Grossly, an irregularly shaped, light tan-pink to whitish mass filled most of the posterior segment of the left eye and was partially attached to the posterior pars ciliaris retinae (Fig. 1). The retina was detached and displaced anteriorly. The vitreous was liquefied. The lens and iris were anteriorly displaced and blood filled the compressed anterior and posterior chambers. Histologically, the mass was a densely cellular neoplasm of neuroepithelial origin that had extensively infiltrated and replaced portions of the retina extending into the subretinal space, causing retinal detachment, degeneration, and atrophy. The neoplastic tissue comprised dense sheets of oval to polygonal cells, with hyperchromatic nuclei, that exhibited 0–4 mitotic figures per ×400 magnification field. In several areas, neoplastic cells formed distinctive rosettes resembling both Flexner–Wintersteiner and Homer–Wright rosettes (Fig. 2). The neoplastic tissue was partially subdivided by vascularized fibrous septa and necrotic in several regions. Small groups of large cells with abundant eosinophilic cytoplasm and relatively small nuclei, resembling hypertrophic lens epithelium or fiber cells, were scattered within the neoplastic tissue. Plasmoid fluid and blood filled the remainder of the subretinal space. The neoplastic tissue outwardly compressed the ciliary processes. The lens exhibited cataractous changes including hyperplasia and hypertrophy of the lens epithelium and hydropic degeneration of lens fibers. The iris was displaced anteriorly and separated from the posterior surface of the cornea by a thin preiridal fibrovascular membrane that was adhered to the anterior surface of the iris and the corneal stroma through a defect in Descemet's membrane (anterior synechia). The corneal epithelium was extensively ulcerated and numerous degenerate inflammatory cells infiltrated the ulcer margins and the underlying corneal stroma. The corneal stroma was edematous and infiltrated by proliferated capillaries and fibroblasts (granulation tissue).
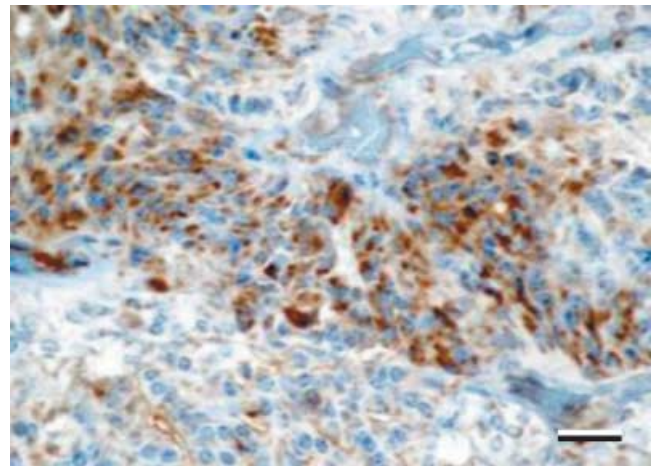
Immunohistochemical analysis revealed that the majority of neoplastic cells, including the rosettes, exhibited diffuse cytoplasmic staining for S-antigen (Fig. 3). In several areas, neoplastic cells also exhibited focal cytoplasmic staining for rhodopsin (Fig. 4). Rod cells in the adjacent retina were uniformly positive for rhodopsin. Neoplastic cells were uniformly negative for GFAP. Localization of staining in the normal llama eye with the antibodies used in this study was consistent with staining reported in other species, confirming the specificity of rhodopsin and S-antigen for photoreceptors in llama eyes.[1–3,21–23]
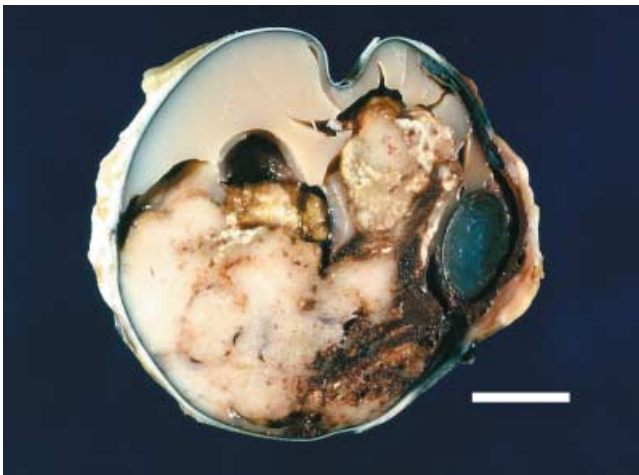
Figure 1. Eye, retinoblastoma, llama. The tumor has filled the posterior segment effecting anterior displacement of the lens. Bar = 1 cm.

Figure 3. Eye, retinoblastoma, llama. Large numbers of neoplastic cells exhibit diffuse cytoplasmic staining for S-antigen. IHC, hematoxylin counterstain, bar = 50 μm.

Figure 2. Eye, retinoblastoma, llama. Neoplastic tissue is densely cellular and focally arranged in distinctive rosettes. H&E staining, bar = 200 μm.
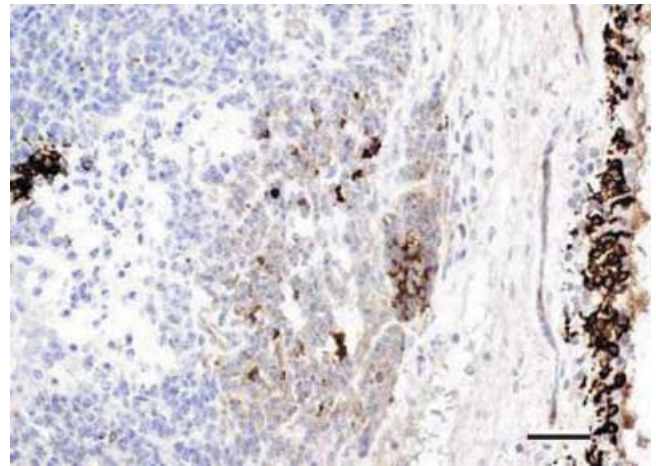
Figure 4. Eye, retinoblastoma, llama. Neoplastic cells exhibit focal cytoplasmic staining for rhodopsin. Rod cells in the adjacent retina are diffusely positive. IHC, hematoxylin counterstain, bar = 100 μm.

The llama did not experience any operative or postoperative complications. Four years after surgery, the llama was healthy, without signs of tumor recurrence or metastasis, and produced one clinically normal cria.

DISCUSSION

The morphologic and immunohistochemical features of this intraocular neuroepithelial neoplasm supported a diagnosis of retinoblastoma. These features included its location within the eyeball, morphologic appearance, formation of classic rosettes, and photoreceptor differentiation as demonstrated by S-antigen and rhodopsin expression. To the authors' knowledge, this is the first reported case of a retinoblastoma in a llama.

As stated previously, there remains considerable debate as to the existence of spontaneous retinoblastomas in animals,

even though one well documented case was described in a dog.[1,2] Neoplasms resembling retinoblastomas have been more frequently diagnosed as medulloepitheliomas.[1,2,8–18] Medulloepitheliomas can be classified as benign or malignant, and teratoid or nonteratoid.[24] One of the previously reported cases in a llama was diagnosed as a malignant teratoid medulloepithelioma.[18] In contrast to the tumor described herein, the previously described tumor appeared to have arisen from nonpigmented ciliary epithelium rather than the retina, and included cartilaginous elements and rosettes that were greater than 60 μm in diameter. The tumor in that study lacked GFAP expression, as did our tumor, but was not examined for S-antigen or rhodopsin expression. It is important to consider that while GFAP is a common feature of retinoblastomas, it is not necessarily unique or an exclusive one. The presence of GFAP within other reported

retinoblastomas may be a result of stromal cell expression within the neoplasm.

Although rare in llamas, intraocular neoplasia should be considered as a differential diagnosis for prolonged and unresponsive ocular disease.[17,25] Enucleation with placement of an intraorbital prosthesis was the procedure deemed appropriate for the clinical signs in this case. The diagnosis of a retinoblastoma was established only after the eye was postoperatively evaluated by gross and microscopic pathology. Retinoblastomas are highly malignant and exhibit aggressive invasive growth in humans. If left untreated and there is central nervous system involvement, the condition can by fatal.[26] With the concerns of secondary tumor metastasis, especially via neural pathways, the determination of optic nerve involvement is important in treatment protocols.[27] Human retinoblastoma patients with optic nerve infiltration are characterized as high risk despite enucleation, warranting adjuvant chemotherapy and orbital radiotherapy.[28] The optic nerve could not be identified or further evaluated in this llama. Optic nerve infiltration was unlikely as the tumor did not recur after 4 years and the llama did not receive post-surgical radiation or chemotherapy. Because of the potential for tumor recurrence, the use of a prosthetic implant in conjunction with removal of a malignant intraocular neoplasm remains controversial. However, it has been employed successfully in dogs and cats and was successful in this case.[29] This is the first reported case of a unilateral retinoblastoma in the eye of an adult llama.

REFERENCES

1. Syed NA, Nork TM, Poulsen GL *et al.* Retinoblastoma in a dog. *Archives of Ophthalmology* 1997; **115**: 758–763.
2. Hogan RN, Albert DN. Does retinoblastoma occur in animals? *Progress in Veterinary and Comparative Ophthalmology* 1991; **1**: 73–82.
3. Donoso LA, Folberg R, Arbizo V. Retinal S antigen and retinoblastoma – a monoclonal antibody histopathologic study. *Archives of Ophthalmology* 1985; **103**: 855–857.
4. Mirshahi M, Boucheix C, Dhermy P *et al.* Expression of photoreceptor-specific S-antigen in human retinoblastoma. *Cancer* 1985; **57**: 1497–1500.
5. Ellias WJ, Lopez MBS, Golden WL *et al.* Trilateral retinoblastoma variant indicative of the relevance of the retinoblastoma tumor-suppressor pathway to medulloblastomas in humans. *Journal of Neurosurgery* 2001; **95**: 871–878.
6. Marcus DM, Brooks SE, Leff G *et al.* Trilateral retinoblastoma: insights into histogenesis and management. *Survey of Ophthalmology* 1998; **43**: 59–70.
7. Jaffey PB, To GT, Xu HJ *et al.* Retinoblastoma-like phenotype expressed in medulloblastomas. *Journal of Neuropathology and Experimental Neurology* 1995; **54**: 664–672.
8. Jenson OA, Kaarsholm S, Prause JU *et al.* Neuroepithelial tumor of the retina in a dog. *Veterinary Ophthalmology* 2003; **6**: 57–60.
9. Langloss JM, Zimmerman LE, Krehbiel JD. Malignant intraocular teratoid medulloepithelioma in three dogs. *Veterinary Pathology* 1976; **13**: 343–352.
10. Wilcock B, Williams MM. Malignant intraocular medulloepithelioma in a dog. *Journal of the American Animal Hospital Association* 1980; **16**: 617–619.
11. Lahav M, Albert DM, Kircher CH *et al.* Malignant teratoid medulloepithelioma in a dog. *Veterinary Pathology* 1976; **13**: 11–16.
12. Eagle RC, Frost RL, Swerczek TW. Malignant medulloepithelioma of the optic nerve in a horse. *Veterinary Pathology* 1978; **15**: 488–495.
13. Riis RC, Scherlie PH, Rebhun WC. Intraocular medulloepithelioma in a horse. *Equine Veterinary Journal* 1990; **10** (Suppl.): 66–68.
14. Bistner SI. Medullo-epithelioma of the iris and ciliary body in a horse. *Cornell Veterinarian* 1974; **60**: 588–593.
15. Schmidt RE, Becker LL, McElroy JM. Malignant intraocular medulloepithelioma in two cockatiels. *Journal of the American Veterinary Medical Association* 1986; **189**: 1105–1106.
16. Blodi FC, Ramsey FK. Ocular tumors in domestic animals. *American Journal of Ophthalmology* 1967; **64** (Suppl.): 627–633.
17. Gionfriddo JR, Gionfriddo JP, Krohne SG. Ocular diseases of llamas: 194 cases (1980–1993). *Journal of the American Veterinary Medical Association* 1997; **210**: 1784–1787.
18. Hendrix DVH, Bochsler PN, Saladino B *et al.* Malignant teratoid medulloepithelioma in a llama. *Veterinary Pathology* 2000; **37**: 680–683.
19. Brooks DE. Orbit. In: *Equine Surgery*, 2nd edn. (eds Auer JA, Stick JA) W.B. Saunders Co, Philadelphia, 1999; 502–505.
20. Patrick DJ, Kiupel M, Gerber V *et al.* Malignant granulosa-theca cell tumor in a 2-year-old miniature horse. *Journal of Veterinary Diagnostic Investigation* 2003; **15**: 60–63.
21. Won MH, Kang TC, Cho SS. Glial cells in the bird retina: immuno-chemical detection. *Microscopy Research and Technique* 2000; **50**: 151–160.
22. Gao J, Cheon K, Nusinowitz S *et al.* Progressive photoreceptor degeneration, outer segment dysplasia, and rhodopsin mislocalization in mice with targeted disruption of the retinitis pigmentosa-1 (Rp1) gene. *Proceedings of the National Academy of Sciences of the United States of America* 2002; **99**: 5698–5703.
23. Montiani-Ferreira F, Fischer A, Cernuda-Cernuda R *et al.* Detailed histopathologic characterization of the retinopathy, globe enlarged (rge) chick phenotype. *Molecular Vision* 2005; **11**: 11–27.
24. Broughton WL, Zimmerman LE. A clinicopathologic study of 56 cases of intraocular medulloepitheliomas. *American Journal of Ophthalmology* 1978; **85**: 407–418.
25. Gionfriddo JR. Ophthalmology. *Veterinary Clinics of North America Food Animal Practice* 1994; **10**: 371–382.
26. Lopes MBS, Horten BC. Central nervous system tumors. In: *Modern Surgical Pathology*, 1st edn. (eds Weidner N, Cote RJ, Suster S *et al.*) W.B. Saunders Co, Philadelphia. 2003; 2059–2120.
27. Barron CN, Saunders LZ, Jubb KV. Intraocular tumors in animals. III. Secondary intraocular tumors. *American Journal of Veterinary Research* 1963; **24**: 835–853.
28. Makimoto A. Results of treatment of retinoblastoma that has infiltrated the optic nerve, is recurrent, or has metastasized outside the eyeball. *International Journal of Clinical Oncology* 2004; **9**: 7–12.
29. McLaughlin SA, Ramsey DT, Lindley DM *et al.* Intraocular silicone prosthesis implantation in eyes of dogs and a cat with intraocular neoplasia: nine cases (1983–1994). *Journal of the American Veterinary Medical Association* 1995; **207**: 1441–1443.