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CASE REPORT

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Intraocular neuroectodermal embryonal tumor in two rabbits

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Abstract

Spontaneous intraocular tumors are rarely reported in rabbits, despite their widespread use as laboratory animals. We describe two cases of intraocular neuroectodermal embryonal tumors, formerly primitive neuroectodermal tumors, in young rabbits. Histologically, both tumors exhibited prominent rosette or pseudorosettes, consistent with the histomorphology seen in human tumors. The neuroectodermal subtype is supported by immunoreactivity for the neuronal markers, SRY-box transcription factor 2, microtubule-associated protein 2, neuronal nuclear protein, and neuron-specific enolase. In one of the rabbits, there was metastasis to the contralateral conjunctiva. Intraocular neoplasms can occur in young rabbits and eyes with refractory disease should be enucleated for clinical management.

KEYWORDS

intraocular neoplasm, medulloepithelioma, neuroectodermal embryonal tumor, PNET, retinoblastoma

1 | INTRODUCTION

Intraocular neuroectodermal embryonal tumors (NETs) are rare and sporadically reported in dogs, cats, horses, birds and llamas.[1–9] As there are no reports of neuroectodermal tumors in rabbits, we describe NETs in two pet rabbits.

Naturally occurring intraocular neoplasms in rabbits are rarely reported despite their widespread use as laboratory models.[10] These include post-traumatic sarcoma, iridociliary and melanocytic tumors and lymphoma.[10] Rabbits inoculated with tumor cells are used as models of retinoblastoma for human studies, but we found no reports of spontaneous NETs.

Primary embryonal tumors occur in the central and peripheral nervous system, and in the eye. These tumors derive from neuroectodermal cells of the neurotube, the primitive progenitor cells of the nervous system.[11] In the eye, these cells give rise to the optic vesicle and later, the optic cup. Neoplastic transformation results in medulloepithelioma or retinoblastoma (if there is retinal differentiation).[11]

Intraocular embryonal tumors are comprised of primitive neuroblasts that form tubules or rosettes with or without luminal ciliary processes.[12] The rosettes are either Flexner-Wintersteiner rosettes with empty lumen, or Homer Wright rosettes with central luminal processes. The two major embryonal tumors are medulloepithelioma and retinoblastoma, and they are histologically similar. Retinoblastomas in humans have either type of rosette plus a mutation in the retinoblastoma (RB1) tumor suppressor gene.[13] Medulloblastoma is similar without the RB1 tumor

suppressor gene mutation. Genetic studies in animals are lacking and separation based on histomorphology alone is arbitrary. Retinoblastomas have Flexner-Wintersteiner rosettes with retinal differentiation based on immunolabeling for retinal markers. Medulloepitheliomas have both types of rosettes or elongated tubules, and lack retinal markers. If there are non-ocular elements (cartilage, striated muscle, neural tissue) the tumor is classified as teratoid medulloepithelioma.[3,4,6,12] There are three known cases that conflict with these criteria in veterinary species: a diagnosis of retinoblastoma was made in a llama and a horse with a tumor exhibiting both types of rosettes and both labeled positively for retinal markers (rhodopsin and Retinal S protein), and a cockatiel was diagnosed with medulloepithelioma with a tumor exhibiting Flexner-Wintersteiner rosettes only.[3,14,15] Until genetic testing is readily available for veterinary species, differentiating these two tumors relies on immunohistochemical properties rather than histomorphology alone. The term intraocular NET is applied here.

2 | MATERIALS AND METHODS

Client consent and use of data was granted from the respective teaching institutions for this study. Case 1 was seen by a boarded veterinary ophthalmologist (CLP) at the Ontario Veterinary College, Health Sciences Center and case 2 was retrieved from the Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW) archive. Each case routinely processed for histopathology and all immunohistochemistry (IHC) was performed at Cornell University, College of Veterinary Medicine (Dr Andrew Miller). The available neuronal markers for rabbit tissues were SOX2 (SRY-box transcription factor 2), MAP2 (microtubule-associated protein 2), NeuN (neuronal nuclear protein), NSE (neuron-specific enolase), vimentin and pancytokeratin (AE1AE3). Specific retinal markers are not available in this species as antibodies are produced in rabbits and can cross react, resulting in false positive results.

2.1 | Clinical presentation

2.1.1 | Case 1

A 3-year-old, female Rex rabbit presented for an intraocular mass and secondary glaucoma of the right eye (OD). Treatment included systemic enrofloxacin and fenbendazole, and topical diclofenac and dorzolamide. After 5 months of treatment the OD was buphthalmic with clinically apparent conjunctivitis, episcleritis, diffuse moderate corneal edema, keratitis, focal black corneal endothelial pigmentation ventrally, a white iridal lesion medially, and black iris pigmentation in the dorso-lateral quadrant. A mature cataract prevented fundic examination. The left eye (OS) was assessed as normal except for a concave optic disc. Dazzle response and direct pupillary light response were absent in OD but present in OS; palpebral reflexes were present in both eyes (OU). Schirmer tear test measured 7 mm/min OU and fluorescein stain negative OU. Intraocular pressure OD was 29–30 mmHg, and 23 mmHg OS. Pre-operative bloodwork was within normal limits and no other anomalies were noted on physical examination. Clinical diagnoses of OD included secondary glaucoma and mature cataract leading to vision loss. The right eye was enucleated, and histopathology performed. Chest

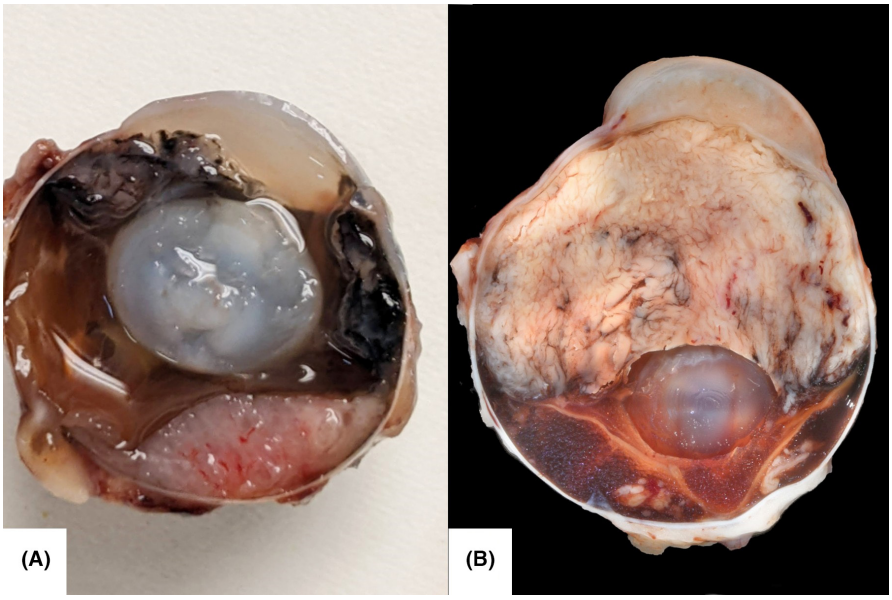

FIGURE 1 Gross images of intraocular embryonal tumors in two rabbits (Case 1: A, Case 2: B). The neoplasms are both grossly pale tan, firm, and expansile. The mass in case 1 (A) spans the choroid, ciliary body, and iris and the mass in case 2 (B) fills the entire posterior segment of the globe.

radiographs were normal 3 weeks post-enucleation and no complications occurred during an ovariohysterectomy 3 months later. There were no clinical complaints at the 5-month follow-up appointment.

Gross findings (Figure 1A): The cornea was diffusely opaque with an irregular surface and the anterior segment was filled with turbid fluid with yellow and red specks. The iris was globally and asymmetrically thickened, the lens was diffusely opaque with multifocal white to gray discoloration, and the choroid was expanded by a pale tan, firm, compressible mass.

Histologic findings (Figure 2A): There was an expansile neoplasm in the choroid between the retinal pigmented epithelium and sclera which extended into the iris, posterior aspect of the cornea and lens, filling the iridocorneal angle. The neoplastic cells formed single-layered Flexner-Wintersteiner rosettes, and often palisaded around small vessels (pseudorosettes). There were occasional Homer Wright rosettes. In other areas there were multilayered pseudorosettes with basally oriented nuclei or broad cords composed of palisading cells. The cells had distinct cell borders with a scant to moderate amount of amphophilic cytoplasm. The nuclei were round, with dispersed chromatin and indistinct nucleoli. There was twofold change in cell and nucleus size (anisocytosis/anisokaryosis) and 42 mitotic figures in 2.37 mm^2 (as calculated for 10, 40× high-power fields, FN 22 mm eyepiece). Approximately 10% of the neoplasm was necrotic. There was exposure keratitis, cataractous changes in the lens and glaucomatous retinal atrophy and retinal detachment.

Immunohistochemistry (Table 1, Figure 3): The neoplastic cells were strongly immunoreactive for SOX2 (diffuse, nuclear) and MAP2 (80%–90% of neoplastic cells, cytoplasmic), moderately immunoreactive for NSE (60%–70% of neoplastic cells, cytoplasmic, apical), faintly immunoreactive for NeuN (5%–10% of neoplastic cells, nuclear), and negative for pancytokeratin and vimentin.

2.1.2 | Case 2

A 4-year-old male rabbit of unknown breed and incomplete medical history presented for an intraocular mass, with a previous history of traumatic eyelid laceration

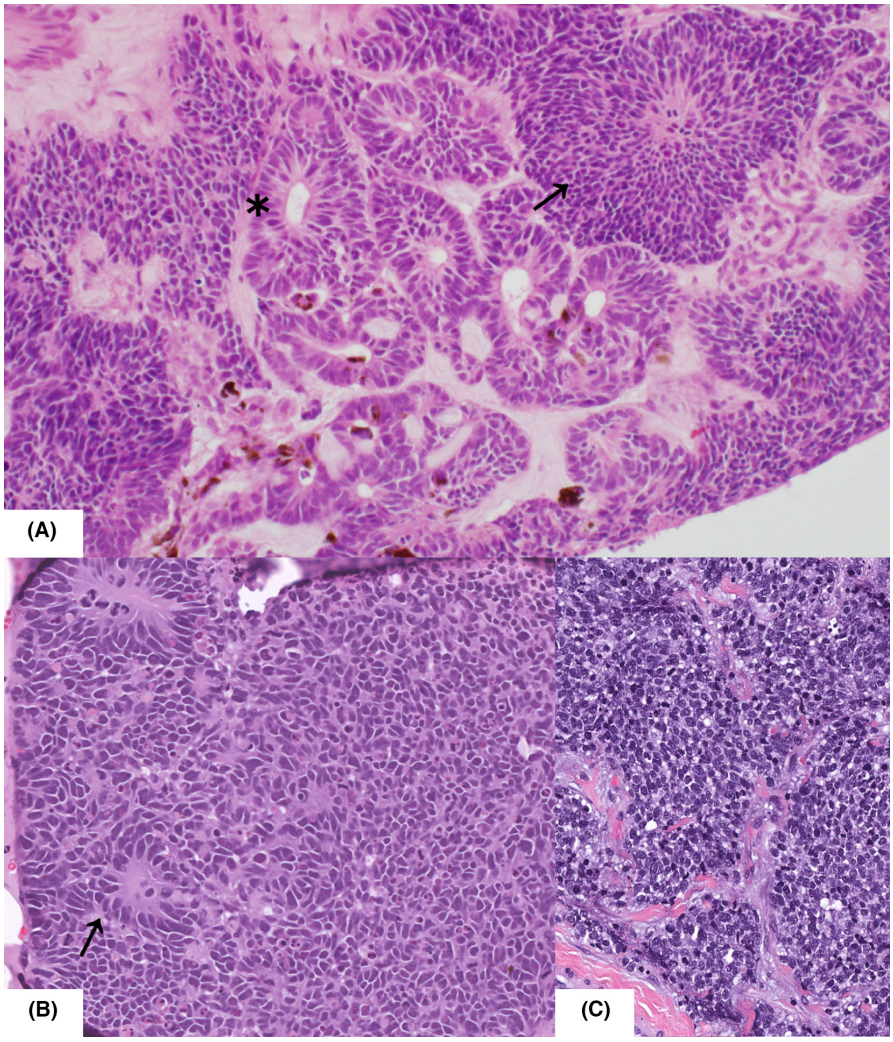

FIGURE 2 Histologic features of intraocular embryonal tumor with neuroectodermal origin in the globe of two rabbits (Case 1: A, Case 2: B, C). Hematoxylin and Eosin, 200× magnification. In case 1, the neoplasm has both Flexner-Wintersteiner (*) and Homer Wright rosettes (arrow). In other areas, there is palisading around small vessels (pseudorosette), or elongated tubules lined by multilayered rosettes (not shown). In case 2, the intraocular tumor displays fewer Homer Wright rosettes (arrow) and is predominantly comprised of sheets of amphophilic, polygonal cells (B). The tissue from the conjunctiva taken 5 months later shows the same population of neoplastic cells, demonstrating metastasis of this neoplasm (C).

TABLE 1 Summary of immunohistochemical staining properties of intraocular embryonal tumor in two rabbits.

IHC marker	Case 1	Case 2	Distribution	Location	Internal positive control
MAP2	+	+	80%	Cytoplasmic	Retina
NSE	+	+	70% of neoplastic cells	Cytoplasmic	Retina
Vimentin	−	+	80% (case 2)	Membranous	Ciliary body, inner plexiform layer of retina
SOX2	+	+	100%	Nuclear	Patchy in retina
NeuN	+	+	5%	Nuclear	Ciliary body
Pancytokeratin	−	−	−	−	Corneal epithelium

Abbreviations: MAP2, microtubule-associated protein 2 (neuron-specific protein); NeuN, neuronal nuclear protein (post-mitotic neuronal marker); NSE, neuron-specific enolase (neuronal marker); Pancytokeratin, AE1AE3 (epithelial cell marker); SOX2, SRY-box transcription factor 2 (multipotential neural stem cell marker); Vimentin, mesenchymal cell marker.

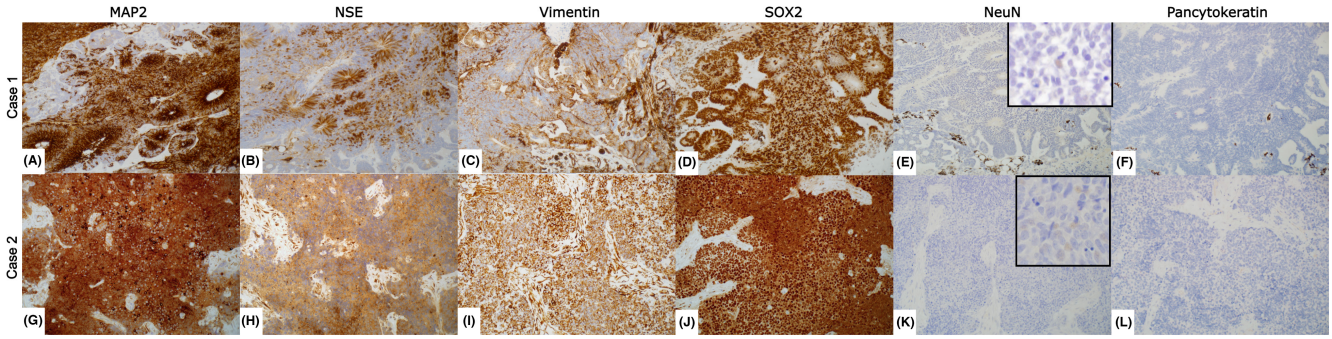

FIGURE 3 Immunohistochemical staining profile of the embryonal tumors (Ocular tumor, Case 1: A–F, Conjunctiva, Case 2: G–L). 200× magnification, inset 400× magnification, Hematoxylin counterstain. Immunoreactivity to MAP2, NSE, Vimentin, SOX2, NeuN, and pancytokeratin was similar in both cases. There is strong, cytoplasmic immunoreactivity to MAP2 in 80% of the neoplastic cells (A, G), moderate, cytoplasmic immunoreactivity to NSE (B, H), with apical bias in case 1 (B). The neoplastic cells were negative for Vimentin in case 1 with positive reactivity in the intracellular space (C) but reactive in case 2 in 80% of the neoplastic cells, with membranous distribution (I). Both cases had strong, nuclear immunoreactivity to SOX2 (D, J) throughout all the neoplastic cells. Immunoreactivity for NeuN was faint, intranuclear and in less than 5% of the neoplastic cells (E, K). The inset is 400× of the faintly positive cells. There was no immunoreactivity to pancytokeratin in either case (F, L).

OS. On ophthalmic examination, OS was buphthalmic with corneal vascularization. The cornea was opaque, and the anterior segment was not visible. Intraocular pressures were within normal limits. Ocular ultrasound showed an intraocular mass with retinal detachment. The OD was unremarkable. The OS was enucleated and submitted for histopathology. Five months later, the rabbit showed signs of difficulty breathing, loss of appetite, and enlarged mandibular lymph nodes. Ophthalmic examination of OD revealed severe, firm, conjunctival swelling and mucoid discharge. The globe OD was unremarkable. A sample of the conjunctiva was submitted for histopathology after euthanasia. Postmortem evaluation was declined.

Gross findings (Figure 1B): The cornea was diffusely opaque. An expansile, pale tan, friable mass filled the entire anterior segment and vitreous chamber, and effaced the uvea and vitreous. The lens was posteriorly luxated and the retina was detached.

Histological findings (Figure 2B): Effacing the anterior uvea circumferentially and expanding and filling 100% of the anterior chamber and 80% of the vitreous chamber was an unencapsulated, non-pigmented, well-demarcated neoplasm forming dense sheets with occasional Homer Wright rosettes. It expanded the mid-corneal paraxial stroma and infiltrated the peripheral cornea and dorsal limbal sclera.

The neoplastic cells had well-defined cytoplasmic boundaries and a moderate amount of amphophilic cytoplasm, irregular round to oval nuclei, coarsely stippled chromatin, and single nucleolus. There was threefold anisocytosis and anisokaryosis. Significant freeze–thaw artifact prevented an accurate mitotic count; there were 5 mitotic figures in three complete high-power fields (0.711 mm^2). Secondary changes in the eye included multifocal corneal ulceration, cataract, retinal detachment and necrosis, and optic nerve gliosis. The vitreous and subretinal space contained abundant glassy

eosinophilic proteinaceous material with free-floating islands of neoplastic cells.

The conjunctival mass collected from OD had a similar population of neoplastic cells in a dense fibrous stroma, similarly arranged in sheets and faint lobules with rare, indistinct pseudorosettes, thus it was considered metastatic from the neoplasm in OS.

Immunohistochemistry (Table 1, Figure 3): Due to significant freeze–thaw artifact of the globe, IHC was performed on the conjunctival mass instead. The IHC staining profile was identical to case 1 with an additional finding that 80% of the neoplastic cells had positive reactivity to vimentin.

3 | DISCUSSION

We report two rabbits with primary intraocular neuroepithelial embryonal tumors (NETs) with histological features of retinoblastoma and medulloepithelioma. The presence of rosettes and pseudorosettes and strong reactivity for Sox2, Map2, and NSE, and weak reactivity for NeuN, supports a diagnosis of the NET.

Distinction between medulloepithelioma or retinoblastoma in these 2 cases was limited by available immunohistochemical antibodies for rabbits. In either tumor type, histomorphology alone was insufficient to definitively distinguish between the two types (Figure 3). Diagnostic criteria for medulloepithelioma in the dog study was based on predominance of pseudorosettes and the lack of immunoreactivity for retinal markers.[8] In these rabbits, case 1 had pseudorosettes and rosettes, and case 2 had rosettes. Thus they do not fit within the scheme proposed by Regan et al.[8]

Both rabbits were approximately 3–4 years of age, which is suggestive of a medulloepithelioma since this is older than the typical age of presentation for retinoblastoma (1–2 years in humans and other animal species). In a study with eight dogs, those diagnosed with retinoblastomas had a mean age of 1.2 years, and those diagnosed with medulloepithelioma had a mean age of 9.1 years.[8] This is consistent with the age distributions in humans, where retinoblastomas occur in infants and medulloepithelioma occur in older children (2–10 years). Case 2 was positive for vimentin whereas case 1 was not. The significance of this is unclear, but similar results have been reported in a llama and cockatiel.[1,3]

Metastasis to regional and distant lymph nodes, lungs and liver was reported in a llama, horse and dogs with medulloepithelioma.[1,7,9] In case 2, there was metastasis to the contralateral conjunctiva. The enlarged lymph nodes and respiratory difficulty leading to euthanasia were suspected to be metastasis but no postmortem evaluation was performed. Case 1 is still alive and disease free. The differing clinical behavior and vimentin staining properties suggests these are separate tumors. However, with limited information, it is difficult to distinguish between variation of the same tumor type versus a truly separate entity.

Retinoblastoma is historically controversial. In nonhuman species, a clear link between RB1 mutations and retinoblastoma is not reported. The rarity of these tumors in animals, despite more identification of RB1 mutations, suggests a different pathogenesis for retinoblastoma.[16]

These tumors were formerly classified under the umbrella term primitive neuroectodermal tumor (PNET). In the human literature, the term PNET was officially abandoned from the diagnostic pathology lexicon in the 2016 World Health Organization (WHO) classification of brain tumors.[17] Whereas previously, PNET referred to a heterogenous group of histologically similar but biologically diverse tumors, the new classification of embryonal tumors is based on molecular profiling.[18] Molecular distinction between medulloepithelioma and retinoblastoma is not available in veterinary medicine. It is unknown if these are distinct entities or representative of a spectrum of the same neoplasm.

Incorporation of molecular information allows for accurate categorization of veterinary tumor entities, which in turn facilitates prediction of biological or prognostic behavior. The distinction between medulloepithelioma and retinoblastoma is not possible until molecular studies are available. These tumors are rare and reports with clinical follow-up are scant.

In veterinary medicine, distinguishing between the two relies on microscopic features and immunohistochemical demonstration of retinal differentiation. It is possible that these represent spectrums of the same entity, rather than two distinct types with histomorphological overlap. Intraocular NETs should be included on the differential diagnosis list for intraocular tumors in young rabbits, especially in the absence of previous ocular trauma. Although not confirmed in this species, these neoplasms could be hereditary, and these animals should be removed from any breeding programs. Histological examination of enucleated eyes is invaluable to further advance our understanding of these rare tumors.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

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