Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2025-2026

Conference #8 — 15 October 2025

CASE I:

Signalment:
1.7-year-old, female spayed, domestic short-hair cat

History:
This animal presented for a large, intraocular mass occupying approximately 80% of the volume of the globe. Corneal opacification and secondary glaucoma were also noted.

Gross Pathology:
On external examination, the eye is notably buphthalmic. The cornea is centrally ulcerated and opaque. On cut surface, a tan, soft mass with multifocal cystic spaces extends from the ventral choroid, protrudes into the anterior and posterior chambers, and abuts and minimally displaces the lens dorsally. The ventral iris is not observed.

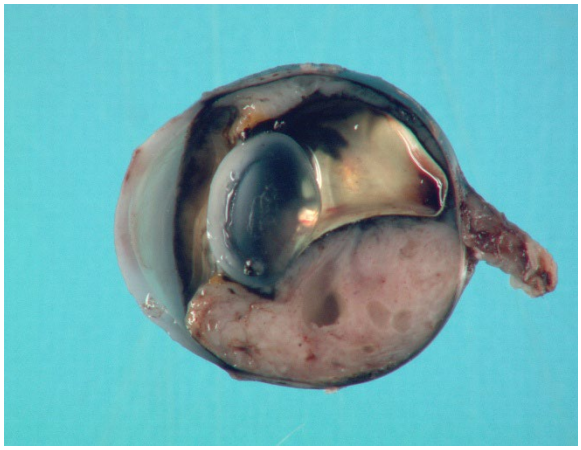

Figure 1-1: Globe, cat. On cut surface, a tan soft mass with multifocal cystic spaces extends from the ventral choroid, protrudes into the anterior and posterior chambers, and abuts and minimally displaces the lens dorsally. The ventral iris is not evident. (Photo courtesy of: National Institutes of Health, https://nih-cbstp.nci.nih.gov/)

Laboratory Results:
N/A

Microscopic Description:
Expanding the anterior uvea, effacing the iris, and markedly expanding the suprachoroidal space is a densely cellular, unencapsulated neoplasm of neuroepithelial cells. Neoplastic cells are arranged in variably dense sheets and as palisading columnar cells surrounding a central lumen (Flexner-Wintersteiner rosettes) or surrounding eosinophilic fibrillar material (Homer-Wright rosettes) surrounded by collagenous and occasionally myxomatous stroma. Neoplastic cells are polygonal to fusiform, have a high nuclear to cytoplasmic ratio, and have distinct cell borders. The nuclei are round to ovoid, finely stippled, and have indistinct nucleoli. There are 16 mitoses in 10 high power fields and anisokaryosis is mild. The neoplastic cells are impinging upon the optic nerve, which has multifocal axonal degeneration and is infiltrated by a population of histiocytes, lymphocytes, and plasma cells, as well as scattered foci of hemorrhage. The retina has multifocal, disorderly components of viable photoreceptor and retinal ganglion cells, dense necrosis, glial scarring, and

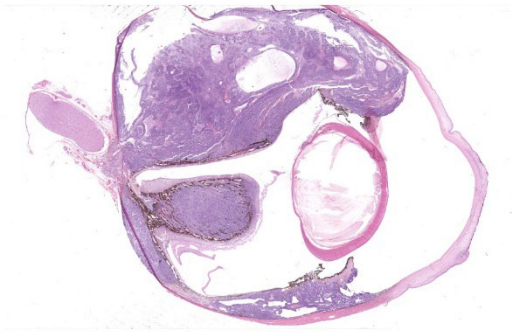

Figure 1-2: Globe, cat. A densely cellular neoplasm expands and effaces the choroid, uvea, and infiltrates the ciliary body and iris. (HE, 10X)

presumed Mueller cells. The retina is diffusely detached and lacks apparent retinal vessels. The retinal pigment epithelial cells have multifocally migrated through to the inner layers of the retina. The cortical lens fibers are moderately liquefied and homogenous and there is posterior migration of the lens epithelium, consistent with cataractous change. The corneal epithelium has marked, segmental ulceration and the outer corneal stroma in this area is mineralized with occasional breaks and is densely compact with fibrosis. Multifocally throughout the corneal stroma are areas of fibrosis, vascularization, and scattered neutrophils.

Contributor's Morphologic Diagnoses:
Eye: Medulloepithelioma, Cataractous change, Ulcerative and neutrophilic keratitis, Stromal fibrosis and mineralization, Diffuse, severe retinal atrophy, Suspect avascular retina, Moderate optic nerve degeneration and necrosis

Contributor's Comment:
Medulloepitheliomas are congenital neoplasms classified as primitive neuroectodermal tumors. They are derived from embryonal neural tissue, the tissue that forms the inner layer of the neural cup. Medulloepitheliomas are categorized into teratoid and nonteratoid forms and further categorized into benign and malignant.[8]

The most common presentation for this tumor is a young animal with a clinically identified intraocular mass. Despite their embryological etiology, medulloepitheliomas have been identified in older animals and age alone is not a disqualifying feature. They have been described primarily in dogs and horses and rarely in other species, including cats, rabbits, and llamas.[2,3,4]

Medulloepitheliomas classically present grossly as white to tan masses that fill the posterior chamber. The hallmark feature of primitive neuroectodermal tumors is the formation of rosettes. This tumor forms both Flexner-Wintersteiner rosettes (characterized by the formation of a central lumen) and Homer-Wright rosettes (characterized by the lack of central lumen and may surround eosinophilic fibrillar material).[10] Importantly, the formation of both rosettes is found in other primitive neuroectoderm tumors and is not unique for medulloepitheliomas. Retinoblastoma is a similar primitive neuroectodermal tumor and must be differentiated histologically from medulloepitheliomas. In retinoblastomas, although they may occasionally have both types of rosettes, Flexner-Wintersteiner rosettes are often the dominant type. While retinoblastomas are common ocular tumors in young children, there are very few accounts of this diagnosis in veterinary species.[5] Characteristically, rosettes in a medulloepithelioma are often large, multi-layered and lined by cuboidal cells arranged in a right angle to the lumen. There is pronounced neuroepithelial tubule formation, a distinguishing characteristic not found retinoblastomas. Medulloepitheliomas have been described more robustly in veterinary species, and the presence of large, multilayered, Flexner-Wintersteiner and Homer-Wright rosettes ultimately led to the diagnosis of medulloepithelioma in this case.

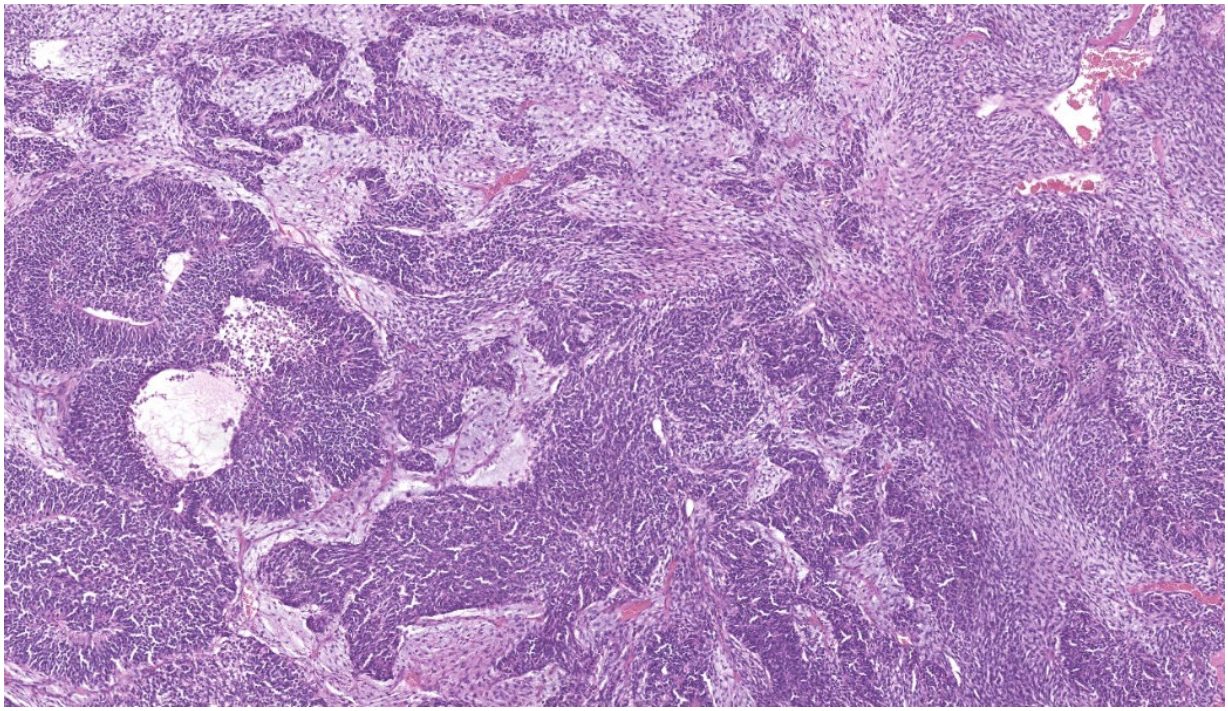

Figure 1-3: Globe, cat. The neoplasm is composed of sheets and trabeculae of palisading neuroepithelial cells which often are separated by loosely arranged myxomatous stroma. (HE, 105X)

Medulloepitheliomas are classified as benign or malignant. Criteria for malignancy set forth by Zimmerman *et al.* include poor differentiation, sarcomatous change, increased mitotic rate or nuclear pleomorphism, and invasion into other structures such as the sclera or uveal stroma.[1] In most veterinary cases with continued follow-up, malignancy was rare. In all cases, enucleation is the preferred treatment method.

Contributing Institution:
National Institutes of Health, Comparative Biomedical Scientist Training Program (CBSTP), 37 Convent Drive, Bldg. 37, Rm. 2002, Bethesda, MD 20892
https://nih-cbstp.nci.nih.gov/

JPC Diagnoses:
Globe, choroid, ciliary body, and iris: Medulloepithelioma.

JPC Comment:
This week's conference was moderated by MAJ Kelsey Fiddes, former Training Officer at the Joint Pathology Center. Two of today's entities (Cases 1 and 2) are new to the Wednesday Slide Conference and provided excellent discussion amongst participants. The conference kicked off with a review of the gross orientation of the eye and how to determine which aspect of the eye contains a lesion (i.e. dorsal, ventral, lateral, medial, etc. A few key features to assess for orientation are the position of the optic nerve, which tends to exit the eye in a ventromedial direction the location of the tapetum lucidum, which is located in the dorsal fundus in dogs, the location of the ciliary arteries (located dorsolaterally and ventromedially from the optic nerve), the dorsal oblique muscle , which is located dorsally and exiting medially from the globe, and the ventral oblique muscle, which is ventrolateral on the globe and exits medially.

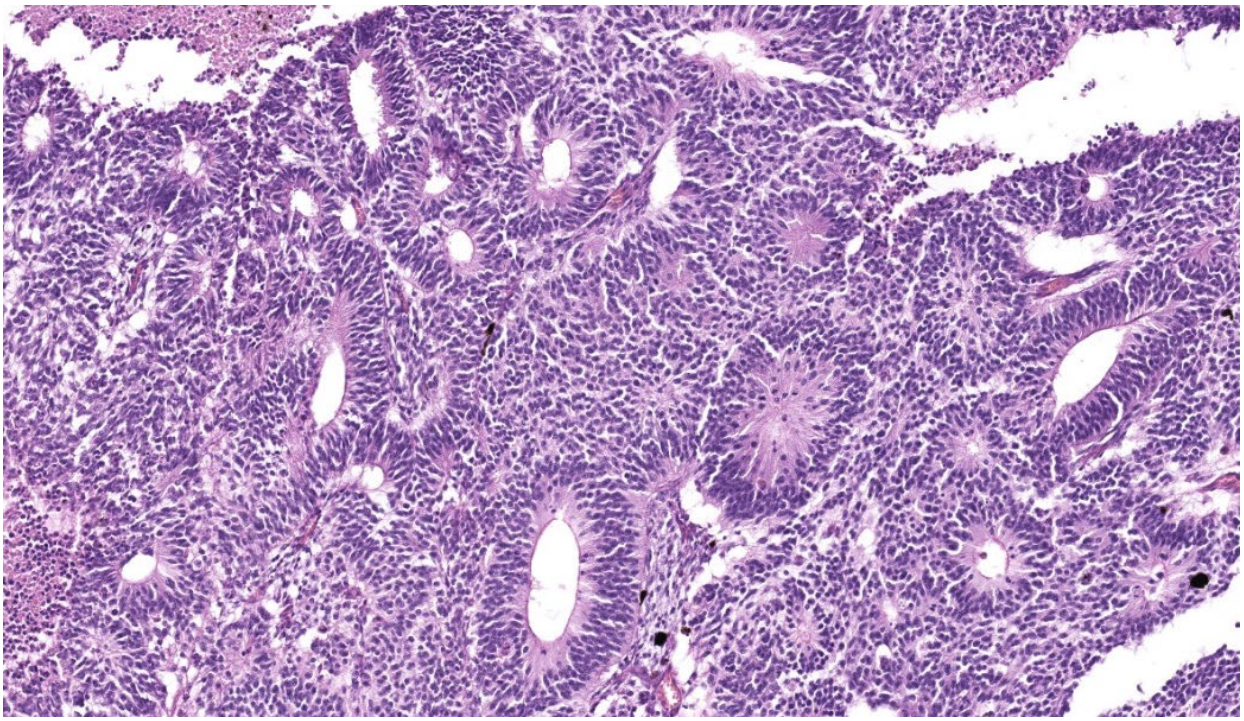

Figure 1-4: Globe, cat. Neoplastic cells form numerous rosettes, some with empty lumina (Flexner-Wintersteiner) and some with lumina that contain neurofilaments (Homer-Wright). (HE, 233X)

Gross preparation of an eye for histopathology was also reviewed and can be summarized as, for standard eye preparation, a vertical cut perpendicular to the ciliary arteries just offset from the optic nerve will provide the best cut for evaluating both tapetal and non-tapetal sides of the choroid. This is especially important for the evaluation of some disease processes, such as tapetal-sparing glaucoma. However, some species require a horizontally oriented cut to ensure that certain structures, such as the pectin in birds or the fovea and macula in some non-human primates, are included in section.

A review of the most pertinent, "board-worthy" basics of feline intraocular neoplasms was performed in the form of a "Jeopardy!" with some feline-centric, pun-based category titles, such as "Meow-lignancy" and "Pathology Purr-view." The major takeaways are: iridociliary tumors can co-express both vimentin and cytokeratin; the most common intraocular malignancy of cats is feline diffuse iris melanoma; neoplastic transformation of lens epithelium, as well as the migration of neoplastic cells to cover the inner circumference of the globe, occurs in feline post-traumatic ocular sarcoma; and the most frequently diagnosed orbital tumor of cats is the feline restrictive orbital myofibroblastic sarcoma.

Lastly, there was much discussion on the extensive secondary changes seen in this eye histologically, including keratitis and panuveitis. It is the opinion of conference participants that these changes were likely secondary to the medulloepithelioma and, as such, favored a traditional JPC morphologic diagnosis in such cases which is limited to the tumor and anatomic locations within the globe. This is not to say that the secondary changes are unimportant or should not be listed, but in true JPC

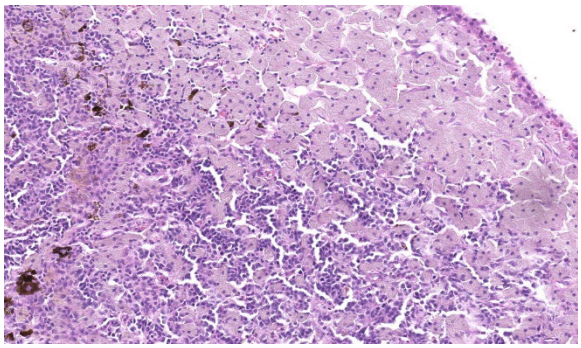

Figure 1-5: Globe, cat. The neoplasm elevates and infiltrates the tapetum lucidum; reflective rodlets are entrapped within the neoplasm. (HE, 305X)

tradition, changes secondary to a tumor are not included in the morphologic diagnosis.

The naming of the medulloepithelioma has a rather storied history. It was first called "carcinome primitif" by French doctors A. Badel and F. Lagrange in their 1892 article titled, "Carcinome primitif des procès et du corps ciliaire" (meaning "Primitive carcinoma of the processes and ciliary body"). These two individuals are credited with the first description of this neoplasm and are considered foundational in ophthalmologic and embryologic pathology. In 1904, a doctor named F. Verhoeff renamed it "teratoneuroma" due to the various tissues seen within the neoplasm in some cases.[9] In 1908, Dr. E. Fuchs decided to call it "diktyoma" after the Greek word "*diktyon*", meaning "net", secondary to the interlacing bands of neuroepithelial cells and meshwork of medullary epithelial cords seen histologically.[9] It wasn't until 1931 that the term "medulloepithelioma" made its way to the scene, coined by Dr. R. Grinker, in reference to the histological resemblance of the tumor to the neuroepithelium of the embryonic neural tube, which is now considered one of the main diagnostic features of this neoplasm.[9]

Intraocular medulloepitheliomas arise from the primitive medullary epithelium of the inner layer of the optic cup. This structure normally develops into the retina, iris, and ciliary body epithelium. This neoplasm most commonly forms in the non-pigmented epithelium of the ciliary body but can rarely originate from the retina or optic nerve. In dogs and cats, intraocular medulloepitheliomas most often follow the playbook and arise from the ciliary body neuroepithelium, but in horses, they tend to go off-book and originate from the optic nerve head.[7] Medulloepitheliomas are classified as either benign or malignant, and teratoid or non-teratoid. The teratoid form, as the name would imply, contains tissues that are not present within the normal eye (e.g., cartilage, bone, brain tissue, or muscle). In the conference case, due to the presence of primitive retinal tissue and neuropil within the neoplasm, conference participants favored a "teratoid" classification for this neoplasm.

Each of the common locations of the intraocular medulloepithelioma represents places where the closure of the optic cup and choroid fissure (also known as the optic stalk fetal fissure) last occur and where the final differentiation of the last multipotent neuroepithelial

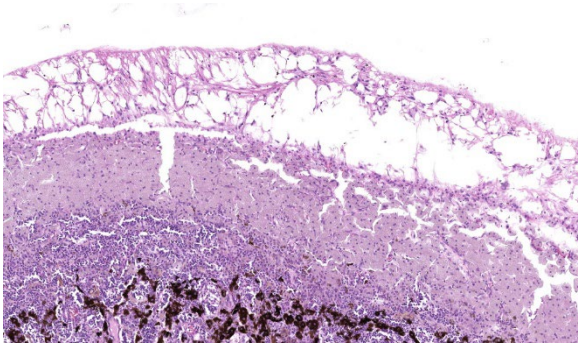

Figure 1-6: Globe, cat. The retina is detached and severely atrophic, with severe loss of nuclei in all layers of the retina, marked loss of nerve fibers and retinoschisis. (HE, 315X)

progenitor cells happens.[6] The optic cup forms from invagination of the optic vesicle, which buds from the developing diencephalon. When the optic vesicle contacts the overlying surface ectoderm, this induces the surface ectoderm to thicken and form the lens placode.[6] Both the lens placode and the underlying optic vesicle begin to invaginate. The invaginating optic vesicle collapses in on its internal space, forming a two-layered optic cup. The outer layer of this optic cup develops into the retinal pigment epithelium (RPE), while the inner layer differentiates into the neural retina, eventually forming the light-sensing rods and cones.[6] The invagination progresses dorsally to ventrally, creating a gap on the ventral surface of the optic cup and stalk called the optic fissure, which is crucial for the development of the optic nerve and other structures. Mesenchymal tissue, mostly from neural crest cells, enters the developing eye through the optic fissure and forms the hyaloid artery, which provides crucial blood supply.[6] The two edges of the optic fissure then fuse together, a process that starts near the optic stalk and moves cranially.[6] This closure is critical for normal

eye development and generally occurs around day 25 in fetal dogs and day 21 in fetal cats under normal conditions. Failure of the optic fissure to close can result in conditions such as coloboma formation.

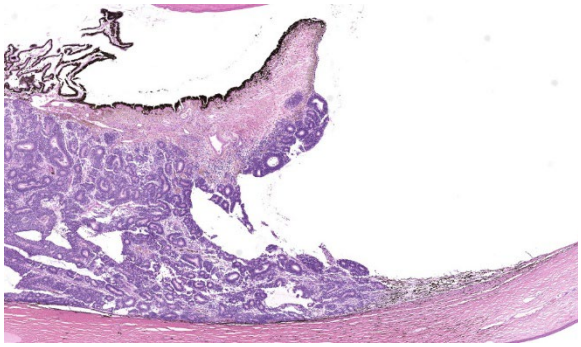

Figure 1-7: Globe, cat. The neoplasms efface the drainage angle and carpets and infiltrates the anterior surface of the iris. (HE, 43X)

Although not considered hereditary, current studies indicate that medulloepitheliomas may be linked to certain genetic mutations that disrupt embryonic development. In some cases, medulloepithelioma formation has been associated with germline mutations in the DICER1 gene, which is involved in microRNA processing (hello, Gen Path).[9] Others may feature somatic mutations in the KMT2D gene, which plays an important role in histone methylation.[9] These genetic changes interfere with the normal differentiation of primitive neuroectodermal stem cells, causing them to develop into a neoplasm instead of their mature, specialized tissues.

References:

1. Broughton WL, Zimmerman LE. A Clinicopathologic Study of 56 Cases of Intraocular Medulloepitheliomas. *American Journal of Ophthalmology*. 1978; 85(3):407-418.
2. Eagle RC Jr, Font RL, Swerczek TW. Malignant medulloepithelioma of the optic nerve in a horse. *Vet Pathol.* 1978;15(4):488-94.
3. Langloss JM, Zimmerman LE, Krehibiel JD. Malignant intraocular teratoid medulloepithelioma in three dogs. *Vet Pathol.* 1976;13(5):343-52.
4. Monk CS, Craft WF, Abbott JR, et al. Clinical behavior of intraocular teratoid medulloepithelioma in two-related Quarter Horses. *Vet Ophthalmol*. 2017;20(6):551-559.
5. Peiffer RLJr, Simons KB. Ocular Tumors in Animal and Humans. Iowa State University Press, 2002, 1st Edition.

6. Rosen D, Mahabadi N. Embryology, Optic Cup. [Updated 2023 May 1]. *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545150/.
7. Salih A, Moore D, Giannikaki S, Scurrell E. Malignant ocular teratoid medulloepithelioma in two cats. *J Comp Pathol.* 2023;201:10-12.
8. Saunders T, Margo CE. Intraocular medulloepithelioma. *Arch Pathol Lab Med*. 2012;136(2):212-216.
9. Tadepalli SH, Shields CL, Shields JA, Honavar SG. Intraocular medulloepithelioma - A review of clinical features, DICER 1 mutation, and management. *Indian J Ophthalmol*. 2019;67(6):755-762.
10. Wippold FJ 2nd, Perry A. Neuropathology for the neuroradiologist: rosettes and pseudorosettes. *AJNR Am J Neuroradiol*. 2006;27(3):488-92

CASE II:

Signalment:
11-month-old Toy Poodle, Female Spayed

History:
The patient presented for left hindlimb lameness which was localized to the left hip. Radiographs were most consistent with a diagnosis of Legg–Calvé–Perthes disease. The patient underwent a femoral head osteotomy, and the tissue was submitted for histopathology.

Gross Pathology:
A photograph of the femoral head taken immediately post operatively. The femoral head is misshapen with a focal region of flattening and indentation adjacent to the ligamentum capitis

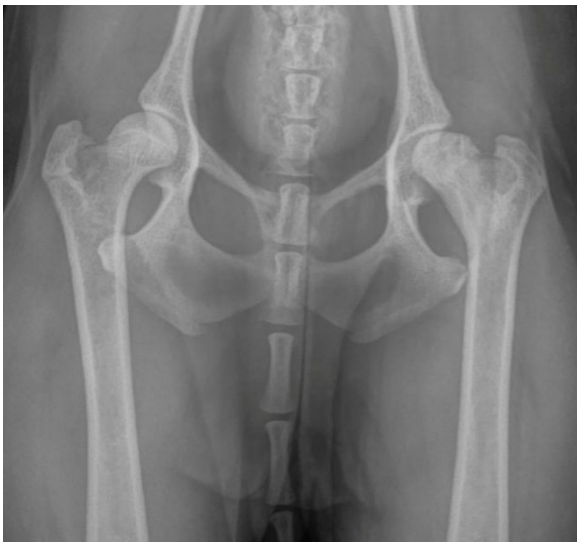

Figure 2-1: Pelvic radiograph, dog. Pre-operative ventrodorsal radiograph of the hips showing bone loss, osteolysis and a severely misshapen left femoral head. (Photograph courtesy of: Murdoch University, www.murdoch.edu.au/theanimalhospital/services/pathol

femoris. Orientation was not provided with the gross image.

Laboratory Results:
Pre-operative VD radiograph of the hips showing bone loss, osteolysis and a severely misshapen left femoral head.

Microscopic Description:
Femoral head: Affecting approximately 30-50% of the epiphysis from the subchondral aspect of the articular cartilage to the physis, there is a focally extensive area of osteonecrosis characterized by bony trabeculae which are irregular and brightly eosinophilic with loss of differential staining. There is loss of both osteocytes within lacunae and osteoblasts lining trabeculae in this region. Along the margins of the necrotic area, multiple bone trabeculae are fragmented and/or have scalloped margins with osteoclasts in Howship's lacunae (osteolysis). The intertrabecular spaces within and at the periphery of the necrotic area contain variably amounts of amorphous eosinophilic matrix (fibrin), occasional neutrophils and mac-

rophages and/or loosely arranged myxomatous to collagenous matrix with variable numbers of plump spindle cells and capillaries (fibroplasia). Occasionally, there are also finer, irregular trabeculae of paler staining or partially mineralized bone (woven bone). Adjacent viable trabeculae are lined by numerous active osteoblasts (remodeling) and are sometimes laced with wavy basophilic lines (resting / resorption lines). The articular cartilage is fragmented (likely artifact).

Contributor's Morphologic Diagnoses:
Osteonecrosis, focally extensive, chronic with fibroplasia and bone remodeling; femoral head.

Contributor's Comment:
The signalment, clinical history, radiographic findings and histopathology are most consistent with a diagnosis of avascular necrosis of the femoral head, also known as Legg-Calvé-Perthes Disease or Osteonecrosis of the femoral head.

Avascular necrosis of the femoral head (ANFH) is a well-recognized condition which occurs most frequently in small breed dogs, including miniature poodles, West Highland White terriers and Yorkshire terriers.[3] This condition is heritable in these breeds and there is evidence to support an autosomal recessive mode of inheritance.[2,3] Most affected individuals present between 3 and 13 months of age with unilateral hindlimb lameness.[2] The condition is bilateral in approximately 12-17% of cases.[2] A significant percentage of dogs have concurrent patellar luxation which can complicate diagnosis, particularly as radiography lacks sensitivity in detecting early cases.[2,7]

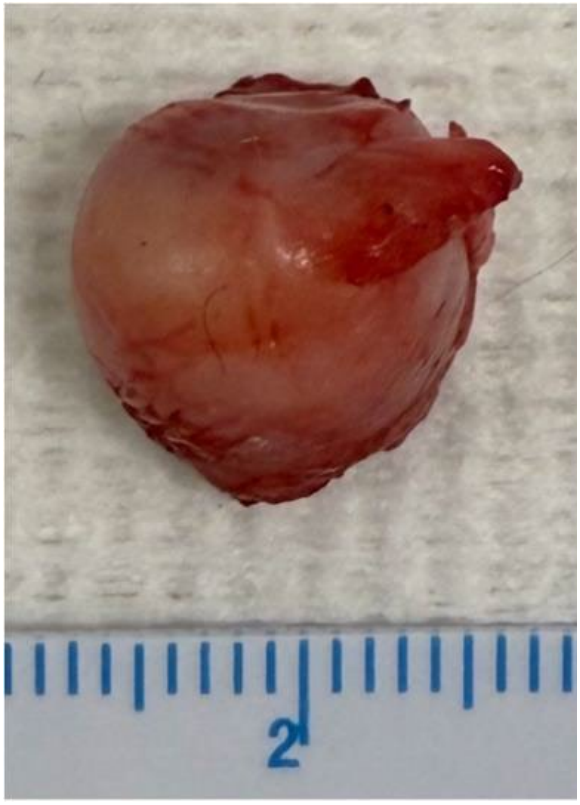

Figure 2-2: Femoral head, dog. The femoral head is misshapen with a focal areas of flattening and indentation adjacent to the ligamentum capitis femoris. (Photograph courtesy of: Murdoch University, www.murdoch.edu.au/theanimalhospital/services/pathology-services)

ANFH is not always a heritable condition. In humans and dogs ANFH can be broadly categorized as non-traumatic or traumatic, and within those categories, as pediatric or adult.[5] Traumatic cases are typically associated with femoral neck fractures; this should be excluded clinically in affected dogs.[3,5] In humans, although most 'non-traumatic' cases of ANFH are deemed idiopathic, there is typically some metabolic disorder which underpins the disease, with alcoholism and steroid administration among the most common in adults.[5] A number of experimental animal models have examined the pathophysiology of

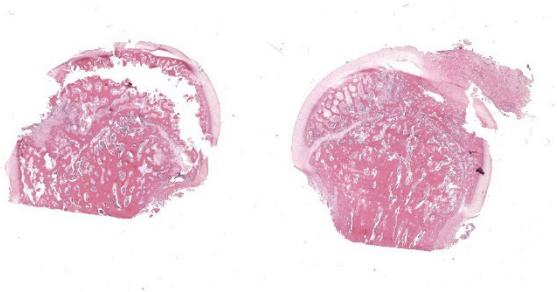

Figure 2-3: Femoral head, dog. Two sections of the femoral head are submitted for examination. (HE, 12X)

osteonecrosis because there remains no consensus on an optimal approach for treatment in humans.[4]

Although ANFH is often labelled 'idiopathic', it is well understood that vascular compromise and subsequent ischemia of the bone are involved in the pathogenesis in both humans and animals.[3,4,5,6] The underlying cause or trigger of vascular compromise is varied, particularly in affected human adults with underlying metabolic disease.[5] In young, small breed dogs with heritable ANFH it is postulated that the development of the vascular supply of the femoral head contributes to the condition.[3] In normal, crossbred dogs the blood vessels supplying the femoral head are gradually incorporated into fibro-osseous canals during development and as such are well protected by the surrounding bone.[3] In Miniature poodles, which are highly susceptible to the heritable, juvenile form of ANFH, the incorporation of these vessels is delayed or does not occur completely.[3] As a result, even a slight increase in intraarticular pressure can result in venous occlusion and subsequent ischemic injury.[3] Joint effusion due to transient episodes of synovitis or minor trauma could trigger the condition in predisposed individuals.[2,3]

The gross and microscopic pathology varies depending on the chronicity of the disease at the time of diagnosis.[1,3] Early on, the shape and structure of the femoral head are grossly and radiographically normal. Histologically, there may be evidence of osteonecrosis predominantly within the subchondral bone at this stage.[1] With chronicity, if the area of necrosis is large enough, the necrotic bone collapses which causes flattening of the femoral head (Figure 1) which eventually results in a degenerative arthropathy.[1,3]

Contributing Institution:
Murdoch University Pathology Services.
www.murdoch.edu.au

JPC Diagnoses:
Femur, epiphysis: Osteonecrosis, chronic, focally extensive, moderate, with osteolysis, woven bone, fibrosis, and bony remodeling.

JPC Comment:
The contributor of this case gives an excellent overview of this condition.

Most of conference discussion was centered on pathogenesis and key histologic features of

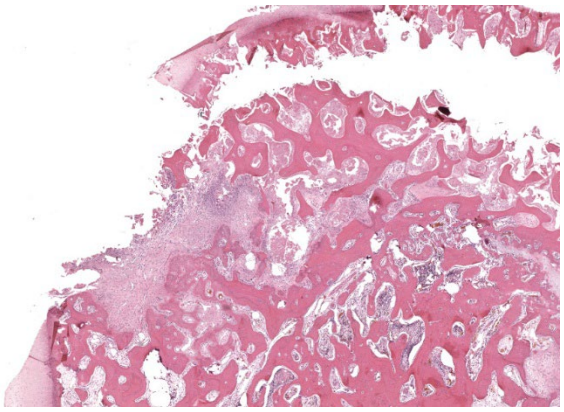

Figure 2-4: Femoral head, dog. There is a focally extensive area of bone loss within the femoral epiphysis. Peripheral to the area of osteonecrosis, trabeculae spaces are filled with variably cellular areas of fibrosis. (HE, 27X)

this condition. This disease can be, as stated by the contributor, either hereditary or secondary to trauma or other ischemic event.[1,3,4,5,6] The hereditary form is most often seen in young, small breed dogs, such as terriers, dachshunds, pugs, Chihuahuas, and toy poodles. When looking at this case, there are a few histologic clues that can assist in determining the age of this animal, which can help with reaching the correct diagnosis. These include the presence of discontinuous physeal cartilage and the horizontal orientation of the bony spicules deep to the physis. In dogs, physes typically close around 10 months of age. Physis closure in Legg-Calves-Perthes disease (LCPD) is typically delayed secondary to ischemia of the physeal cartilage and the subchondral bone, which can result in a malformed or improperly angled femoral head.[1] Additionally, in young animals, the bony spicules that extend into the metaphysis from the developing stages of the growth plate are oriented vertically (perpendicular) to the growth plate to facilitate the elongation of the bone. In older animals that have closed physes, the bone below the growth plate becomes oriented horizontally (parallel) to the growth plate, effectively "capping" it to facilitate closure of the physes. In this case, the bone has become horizontally oriented below the growth plate, indicating that this animal should have closed physes at this stage, but the presence of and discontinuity of the lingering growth plate indicate that this young animal is likely experiencing delayed physeal closure.

There are three recognized grades of LCPD and include: Grade 1, defined as necrosis of bone with empty lacunae, lack of viable bone marrow, and normal articular chondrocytes.

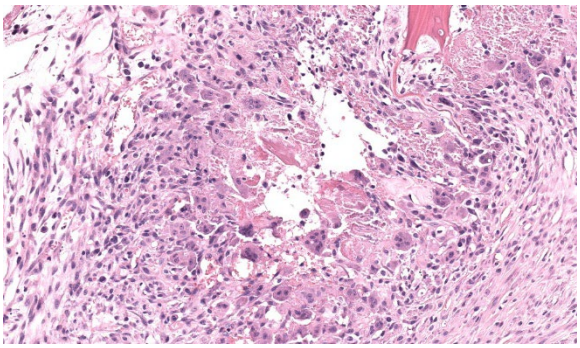

Figure 2-5: Femoral head, dog. In areas of osteonecrosis, fragmented and hypereosinophilic fragments of necrotic bone are surrounded by numerous macrophages, osteoclasts, and cellular debris against a background of fibroblasts and maturing collagen. (HE, 312X)

Grade II, characterized by cracks in the articular cartilage, subchondral bone collapse, and flattening of the femoral head with re-vascularization coming in from the periphery of the epiphysis +/- granulation tissue development; and Grade III, in which the articular cartilage is highly deformed with folds and cracks and the femoral head loses its shape.[1]

In the early stages (Grade I) of LCPD, the articular cartilage is spared because of its avascular nature.[1,3,5] The subchondral bone, however, will become necrotic, and there is frequently a characteristic "tideline" of hypereosinphilia between the unaffected articular cartilage and the affected subchondral bone.[3,5] As the condition progresses, however, the joint is subjected to altered conductive forces from abnormal weight-bearing, which places excessive pressure upon the articular surface and ultimately results in articular cartilage loss.[1,5] This results in both the flattening of the femoral head from mechanical collapse and in asymmetrical growth between the affected limb and unaffected limb, as this disease is usually unilateral.[5] Additionally, secondary to the altered conductive forces through the joint,

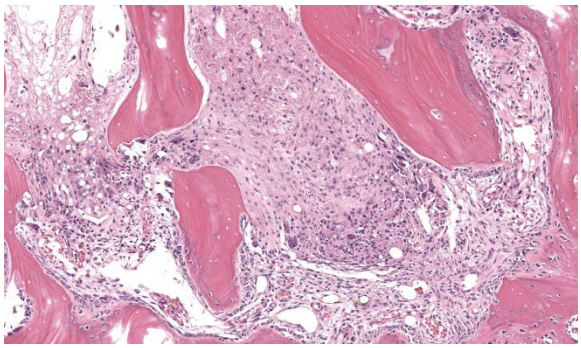

Figure 2-6: Femoral head, dog. Peripheral to the areas of osteonecrosis, there is loss of osteoclasts lining remnant bony trabeculae, and intertrabecular spaces contain abundant variably mature fibrous connective tissue populated with fibroblasts, macrophages, and scattered osteoclasts. (HE, 202X)

new woven bone begins proliferating in an attempt to re-stabilize the bone and adjust for the altered force conduction.[1] This woven bone is usually seen flanking either side of an area of lamellar bone to create a "sandwich" effect.

Differentiating necrotic bone from normal bone can be challenging, but one of the key features to look for is the loss of osteocytes from lacunae.[5] Osteocytes either become pyknotic and hypereosinophilic or disappear entirely, and you can best convince yourself this is a real change (and not just a staining artifact) by comparing an area of suspected necrosis to an area of less affected or normal bone where the osteocytes will still be in their lacunae and have a nice, basophilic nucleus. Another useful way to determine osteonecrosis is to assess the bone marrow. In necrotic bone, there is usually histologic loss of marrow elements secondary to ischemia, which will manifest as a loss of differential staining with retention of architecture within the marrow.

References:

1. Aguado E, Goyenvalle E. Legg Calvé Perthes disease in the dog. *Morphologie*. 2021;105:143-147.
2. Cardoso CB, Rahal SC, Mamprim MJ, Oliveira HS, Merlchert A, Coris JGF, dos Reis Mesquita L. Avascular necrosis of the femoral head in dogs-retrospective study. *Acta Scientiae Veterinariae*. 2018;46:5-5.
3. Craig LE, Dittmer KE, Thompson KG. Bones and joints. Jubb, Kennedy & Palmer's pathology of domestic animals. 2015;1:16-163.
4. Fan M, Peng J, Qin L, Lu S. Experimental animal models of osteonecrosis. *Rheumatology International.* 2011;31:983-994.
5. Guerado E, Caso E. The physiopathology of avascular necrosis of the femoral head: an update. *Injury*. 2016;47:S16-S26.
6. Kerachian MA, Harvey EJ, Cournoyer D, Chow TY, Séguin C. Avascular necrosis of the femoral head: vascular hypotheses. *Endothelium*. 2006;13:237-244.
7. Mapuvire T. Legg-Calvé-Perthes Disease in the Dog: A Case-Based Review. *South African Veterinary Association*. 2023.
8. Vasseur PB, Foley P, Stevenson S, Heitter D. Mode of inheritance of Perthes' disease in Manchester terriers. *Clinical Orthopaedics and Related Research*. 1989;244:281-292.

CASE III:

Signalment:
17-year-old, female (spayed), domestic long hair, Felis catus, feline.

History:
Patient presented with uveitis OU, OD greater than OS. Significant corneal edema and increased intraocular pressure (IOP) recorded

OD. Uveitis seemed to respond to medical therapy, but IOP did not. Enucleation performed OD and submitted for determination of etiology of uveitis.

Gross Pathology:
Intact globe OD submitted with no gross evidence of intraocular neoplasm.

Laboratory Results:
N/A

Microscopic Description:
Eye: Overlying the corneal endothelium, anterior and posterior iris, portions of the lens epithelium, and the inner surface of the retina, as well as effacing and replacing the ciliary body, and occluding the drainage angle is an unencapsulated, densely cellular, infiltrative, neoplasm composed of epithelial cells arranged in broad dense cords on a moderate fibrovascular stroma. Neoplastic cells are polygonal with distinct cell borders, pronounced intercellular bridging, a moderate amount of pale eosinophilic cytoplasm, and irregularly round to vesiculate nuclei, with up to three distinct nucleoli. Anisocytosis and anisokaryosis is moderate. The mitotic rate is high with up to 12 mitotic figures per ten 40x HPF. Multifocally, neoplastic cells exhibit squamous differentiation. Near the optic nerve, the neoplastic cells invade the vascular and fibrous tunics, elevating and dissecting beneath the retinal pigment epithelium under a detached and coiled degenerate retina. At the caudal interior surface of the globe, there are numerous neutrophils admixed with abundant eosinophilic cellular and karyorrhectic debris and neoplastic cells infiltrate into the retinal vasculature.

Contributor's Morphologic Diagnoses:
Eye: Squamous cell carcinoma, domestic long hair, *Felis catus*, feline.

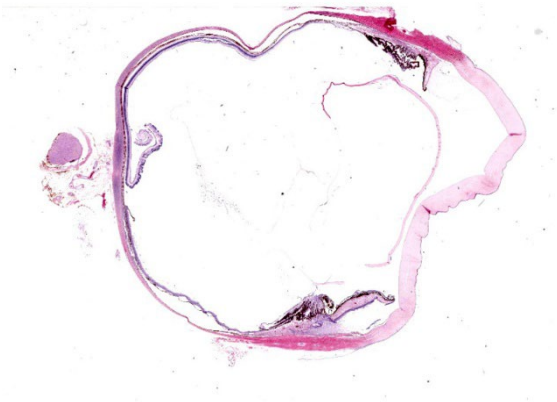

Figure 3-1: Globe, cat. At subgross magnification, the globe appears subjectively enlarged with thinned fibrous tunics. Most of the lens is missing, with only the anterior capsule in section. (HE, 7X)

Contributor's Comment:

Generally, neoplasms within the globe may be (1) primary (arising from one of the intraocular tissues), (2) secondary (invading the globe from an adjacent tissue), or (3) metastatic (arising via hematogenous dissemination of a distant malignancy).[2] Many ophthalmic tumors are histologically benign but are locally invasive and, within the confined space of the eye or orbit, can produce significant tissue distortion.[5]

Surgical definitions which may be relevant when communicating with clinicians include: *evisceration*, which is the removal of the cornea and the internal contents of the eye while leaving the scleral shell of the globe and eye muscles behind; *enucleation*, which is the removal of the entire globe while leaving muscles that control eye movement and other orbital contents intact; and *exenteration*, which is the total removal of the globe and all surrounding tissues including eyelids, muscles, nerves and fatty tissues from the orbit.

Primary intraocular neoplasia. Uveal melanocytic neoplasia ("diffuse iris melanoma" in

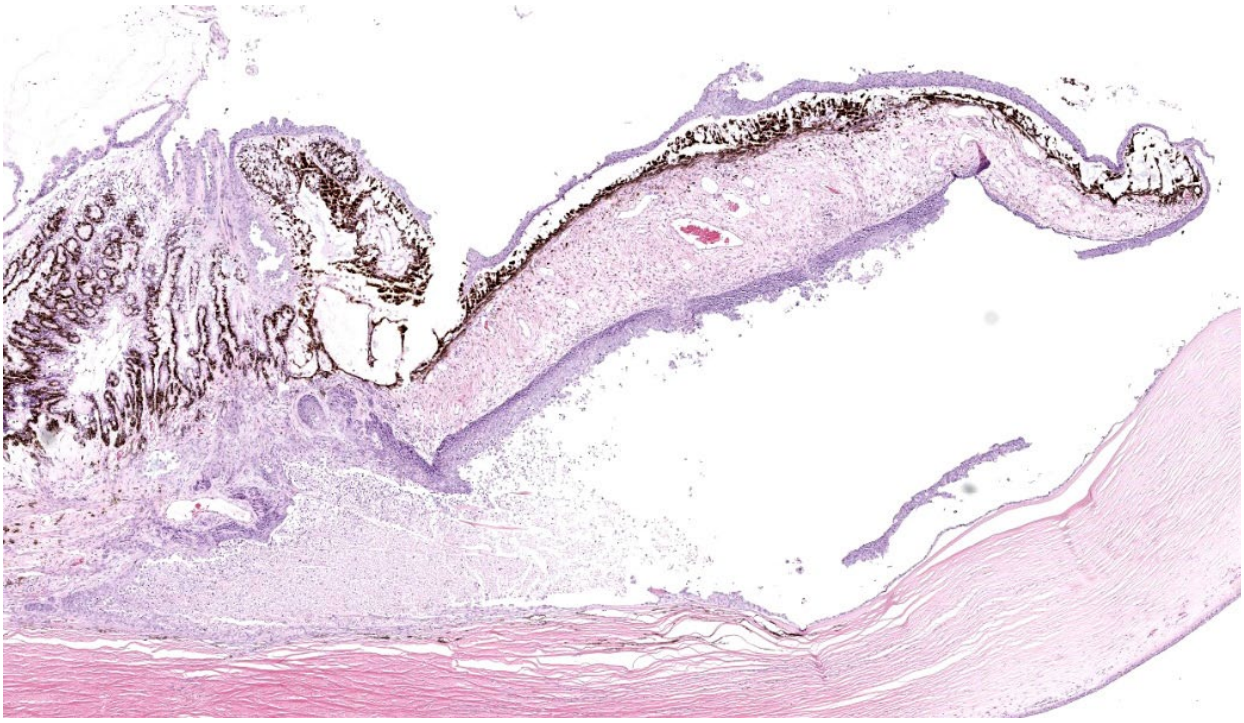

Figure 3-2: Globe, cat. The choroid and ciliary body are expanded and infiltrated, the drainage angle is effaced, and both the anterior and posterior aspects of the iris are carpeted by a moderately cellular epithelial neoplasm. (HE, 55X)

cats and "canine anterior uveal and epibulbar melanoma/melanocytoma" in dogs) is the most common primary intraocular neoplasm, occurring roughly three times as frequently as ciliary body epithelial neoplasms in dogs and ten times as frequently as ciliary body epithelial neoplasms in cats.[1-2,5] This neoplasm typically originates on the anterior surface of the iris, producing multifocal areas of pigmentation with a high potential for local invasion into the ciliary body and the iridocorneal angle.[5] Common presenting clinical signs in affected cats includes hyperpigmentation of the iris, pupillary deficits, buphthalmia resulting from secondary glaucoma, and evidence of uveitis.[5] Extension posteriorly to affect the choroid is rare.[5] Anterior uveal melanocytomas and melanomas may extend along the anterior or posterior scleral emissaria into and through the sclera to involve the periocular tissues, and the optic nerve provides routes of egress for neoplasms in the posterior pole, but extraocular extension does not always correlate with a poor prognosis.[2] Only malignant uveal melanomas and schwannomas in dogs and diffuse iris melanoma and ocular sarcomas in cats carry significant metastatic risk.[2] The regional lymph nodes, liver, and lungs are the principal sites for melanoma metastasis.[5] Other primary intraocular neoplasms are uncommon and include posttraumatic sarcomas of cats and rarely other species such as the rabbit, schwannomas of blue eyed dogs, astrocytomas, and primary neuroectodermal neoplasms including medulloepitheliomas and retinoblastomas in varied animal species.[2] With few exceptions, the majority of primary intraocular neoplasms remain restricted to the globe and timely enucleation is curative.[2] As the globe lacks lymphatics, metastasis from the intraocular tissues will be hematogenous and occurs either through the scleral venous

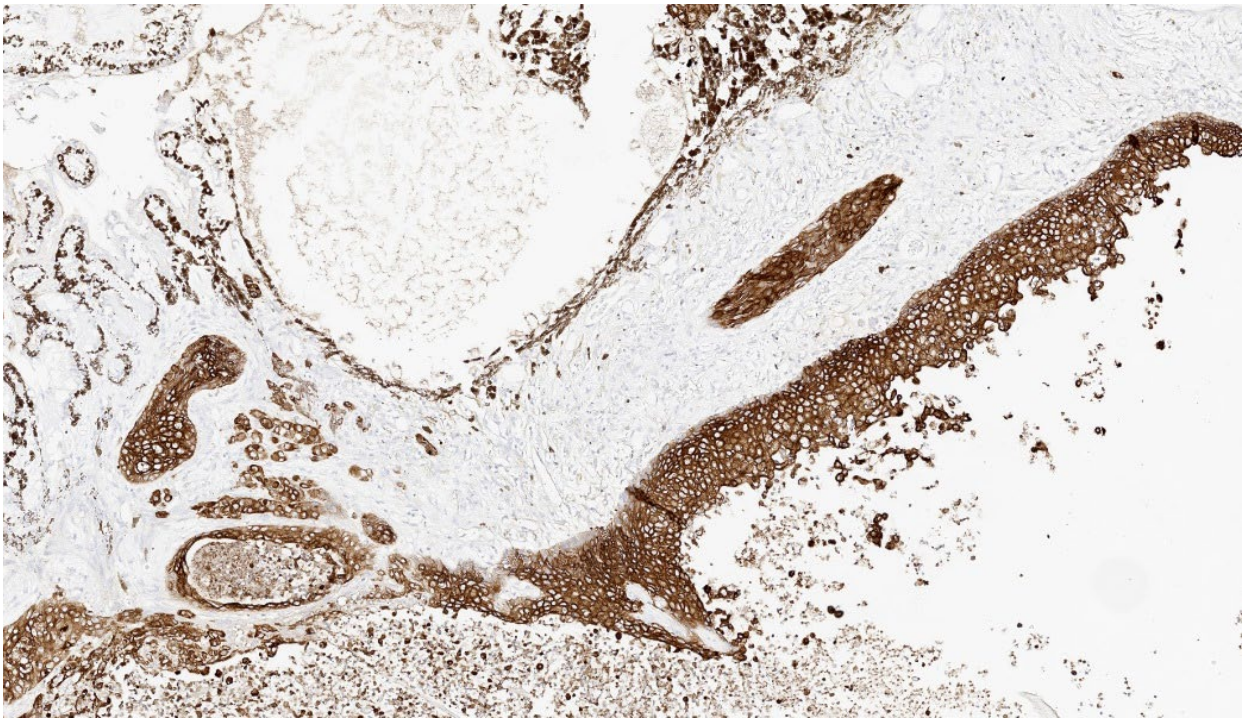

Figure 3-3: Globe, cat. Neoplastic epithelial cells demonstrate strong cytoplasmic positivity for cytokeratin. (anti-AE1/AE3, 55X)

plexus subsequent to invasion of the trabecular meshwork and sclera, or tumor invasion of the uveal and retinal vasculature.[1-2]

Secondary intraocular neoplasia. Aggressive malignancies of adjacent tissues may invade the eye and uvea through the cornea, sclera, or optic nerve.[2] Scant descriptive literature is available; secondary neoplasia is most commonly encountered with squamous cell carcinoma of cow, horse, and cat, and nasal adenocarcinomas with orbital extension in dogs.[2,4] A myxosarcoma involving the optic disc was most likely an extension from the orbit and optic nerve.[2] Meningiomas of the optic nerve may extend through the lamina cribrosa into the globe.[2] Other feline orbital neoplasms include zygomatic osteoma, parosteal osteoma, osteosarcoma, fibrosarcoma, undifferentiated sarcoma, and rhabdomyosarcoma.[4]

Metastatic intraocular neoplasia. It is not surprising that the extensive vascular network of the uvea renders it a prime site for metastatic disease from primary malignancies elsewhere; incidence of metastasis to the eye is less than primary uveal neoplasms but more frequent than implied by the scant literature as the majority of animals dying of widespread metastatic disease are not subjected to thorough ophthalmic clinical, postmortem, and histopathologic examinations.[2] Intraocular lymphosarcoma is the most common metastatic neoplasm in the dog and cat, with shared clinical and histopathologic presentations.[1,2,4] In dogs, these neoplasms occurred bilaterally, predominantly in the anterior uvea, and were diffuse large B-cell, T-lymphoblastic, peripheral T-cell not otherwise specified, and lymphocytic B-cell lymphomas.[1] In cats, feline leukemia virus (FeLV)-associated T-cell lymphoma was the most common.[1] Mammary carcinoma was the second most common ocular metastatic neoplasm in bitches, with a predominantly unilateral involvement of the

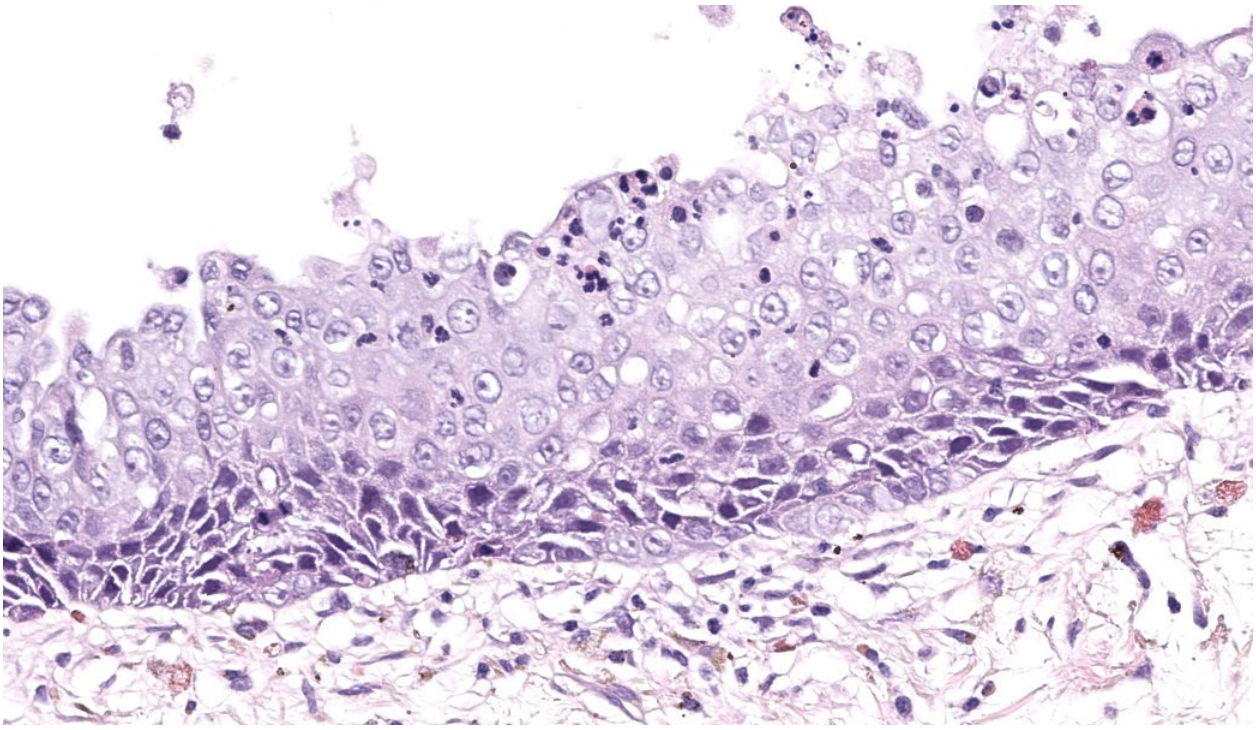

Figure 3-4: Globe, cat. On the anterior surface of the iris, neoplastic squamous epithelium vaguely recapitulates normal maturation with a basal-like layer and multiple layers of additional non-keratinizing squamous epithelium on top. Few neutrophils infiltrate the neoplasm. (HE 730X).

uveal tract.[1] Clinically and by gross examination, intraocular lymphosarcoma is a masquerader and may manifest as unilateral or bilateral uveitis, keratitis, retinal detachment, intraocular hemorrhage, and glaucoma, with or without observable proliferative lesions.[2,4] Not uncommonly, the ocular manifestations may be the presenting sign of occult systemic disease and globes are frequently enucleated due to complicating factors but with a suspicion of intraocular neoplasia.[2,4] Typical ocular presentation of lymphosarcoma in cats is a nodular iridal mass.[3] The question "does unicentric intraocular lymphoma exist" often has been asked, and the response is "probably not," with disease developing elsewhere over time.[2] Additional feline tumors that are known to metastasize to the eye include fibrosarcoma, squamous cell carcinoma, mammary adenocarcinoma, uterine adenosarcoma, and adenocarcinomas of undetermined origin.[4] In cats, following lymphoma, pulmonary and squamous cell carcinomas were the most common multicentric/metastatic neoplasms of the eyes.[1] Pulmonary carcinoma is unusual in its capacity to colonize the vascular endothelium of choroidal retinal arteries.[4] Individual cases of cholangiocarcinoma, hemangiosarcoma, and chemodectoma in the dog, as well as individual cases of mammary gland cribriform carcinoma, salivary gland carcinoma, and histiocytic sarcoma in the cat have been reported.[1]

Conclusion. It is a common belief among specialists in veterinary and human ocular pathology that squamous cell carcinoma cannot originate from within the globe. (J.S. Estep, personal communication; December 26, 2019) Therefore, finding squamous cell carcinoma within the globe of a living patient should prompt the pathologist to warn clinicians to

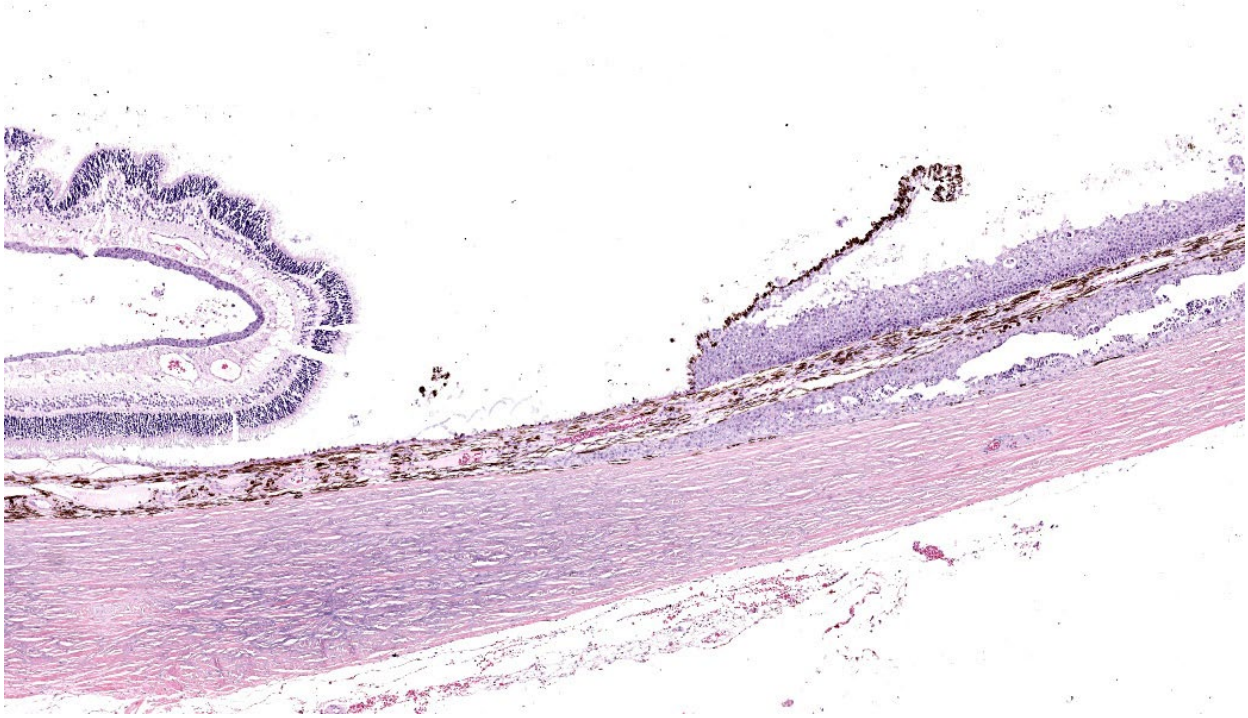

Figure 3-5: Globe, cat. At the back of the eye, the neoplasm infiltrates the choroid and beneath the retinal pigmented epithelium (right), and the retia. (HE 730X).

perform a thorough search for the primary neoplasm elsewhere.

Contributing Institution:
Texas Veterinary Pathology Associates https://texasvetpath.com/index.htm

JPC Diagnoses:
Globe: Metastatic carcinoma.

JPC Comment:
This case contributor gives a thorough review of intraocular neoplasms in cats, touching on many major points of discussion during review of this case. Conference participants were readily able to reach a diagnosis of metastatic carcinoma, but not all were convinced that this was a metastatic squamous cell carcinoma (SCC) due to the lack of dyskeratosis within neoplastic epithelial cells, coupled with the lack of a primary mass found during workup. The prominent intercellular bridging between the neoplastic cells was noted by all, which can be a major feature of SCC; and squamous cell carcinomas are common tumors of the feline head; however other participants felt strongly that they could not rule out a carcinoma of other origin based on histology alone. For this reason, a morphologic diagnosis of "metastatic carcinoma" was ultimately favored by participants in this case due to the lack of clear-cut evidence of a squamous cell carcinoma on the H&E.

There was no argument to be found on whether this was primary or metastatic, as the histologic evidence was strongly supportive of a metastatic process (i.e., the neoplasm primarily found within the highly vascular choroid and uvea, intravascular neoplastic cell emboli, etc.). The secondary changes in the eye were also discussed and it was concluded that this eye had glaucoma secondary to the neoplasm, evidenced by the retinal ganglion cell degeneration and loss with tapetal sparing,

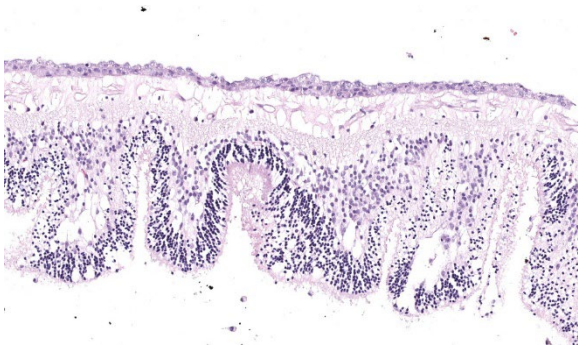

Figure 3-8: Globe, cat. The detached area of the retina demonstrates marked atrophy with almost total loss of ganglion cells, loss of nuclei in the inner and outer nuclear layers, and loss of nerve fibers and photoreceptors. The inner surface of the retina is carpeted with multiple layers of neoplastic squamous epithelium. (HE 307X).

occlusion of the drainage angles by both the neoplasm and inflammation, buphthalmia (enlarged globe, attenuated and degenerative corneal epithelium, scleral thinning), and perivascular edema of the aqueous veins that drain the trabecular meshwork of the drainage angle.

During conference, yet another opportunity arose to briefly discuss common intraocular neoplasms of cats. Key nuggets from this discussion include the fact that cats can develop primary uveal schwannomas similar to those seen in blue-eyed dogs, the most common metastatic neoplasm of the eye in cats is lymphoma, and most other metastatic tumors to the eye in cats are carcinomas that most often affect the posterior uvea.[1,2]

In keeping with her "games" theme, MAJ Fiddes put participants through a few rounds of "I Spy" during this case, focused entirely on the pancytokeratin (AE1/AE3) immunohistochemical stain run on this case and a few issues spotted therein. The first issue was that, while the neoplastic cells had strong cytoplasmic immunoreactivity to pancytokeratin, the retinal pigmented epithelium (RPE) was diffusely unreactive. As it turns out, RPE is specifically reactive for CK8/18, and the pancytokeratin cocktail used by the JPC does not include these. Instead, CK8/18 is offered as a stand-alone IHC here, and its absence in the pancytokeratin would explain why the RPE was not reactive. The second issue was that the neural fibers of the optic nerve had diffuse reactivity to pancytokeratin. That's weird, why would neural tissue be reactive to cytokeratins? Well, as fate would have it, it's not. Pancytokeratin and GFAP are cross-reactive. GFAP is not cross-reactive with most individual cytokeratins, but is to pancytokeratin, making neural tissue appear immunoreactive to AE1/AE3![3] Between AE1 and AE3, it is the AE3 component specifically that contributes to this cross-reactivity.[3] This has been demonstrated in brain and glial tissues, as well as in some retroperitoneal schwannomas. As such, interpretation of cytokeratin expression in neural tissue, including neoplasms of neural origins, should be cautiously interpreted.[3] This "I Spy" game was a poignant reminder that pathologists should scrutinize IHCs and ensure good external and internal control prior to reading them out, and knowledge of what reacts to what can be critical to appropriate interpretation.

References:

1. Bandinelli MB, Viezzer Bianchi M, Wronski JG, et al. Ophthalmopathologic characterization of multicentric or metastatic neoplasms with an extraocular origin in dogs and cats. *Vet Ophthalmol*. 2020;23(5):814-827.
2. Grahn B, Peiffer R, Wilcock B. Intraocular neoplasia. In: *Histologic Basis of Ocular Disease in Animals*. 1st ed. Hoboken, NJ: Wiley-Blackwell; 2019:409,433,435-436.

3. Kriho VK, Yang HY, Moskal JR, Skalli O. Keratin expression in astrocytomas: an immunofluorescent and biochemical reassessment. *Virchows Arch.* 1997;431(2):139-47.
4. La Croix NC. Ocular manifestations of systemic disease in cats. *Clin Tech Small Anim Pract*. 2005;20(2):121-128.
5. Willis AM, Wilkie DA. Ocular oncology. *Clin Tech Small Anim Pract*. 2001;16(1):77-85.

CASE IV:

Signalment:
10-week-old male mixed-breed puppy (*Canis familiaris*, dog)

History:
The body of a 10-week-old mixed breed puppy was received for post-mortem examination. The pup died unexpectedly on a commercial airline flight from the Dominican Republic to Canada. The pup was travelling in a cage in the passenger cabin.

Gross Pathology:
There was mild generalized icterus of mucous membranes and soft tissues. The spleen was enlarged and meaty in texture on cut section. The liver was pale. The renal cortices were dark red-grey, and the urinary bladder contained red-tinged urine.

Laboratory Results:
Fresh-frozen lung was submitted to the Vector-Borne Disease Lab at North Carolina State University for PCR analysis for *Babesia, Anaplasma, Bartonella, Ehrlichia, Rickettsia,* and Hemotropic *Mycoplasma spp.* PCR was positive for *Babesia vogeli*.

Microscopic Description:
Alveolar septa are mildly thickened and hypercellular, and contain increased numbers of macrophages, rare neutrophils, and small

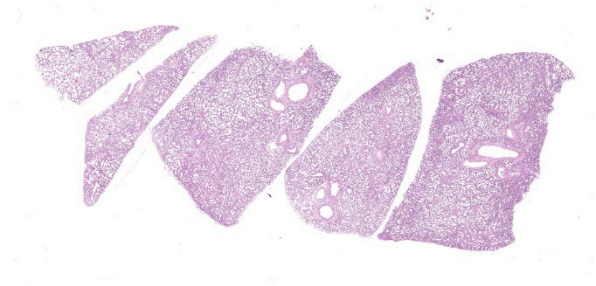

Figure 4-1: Lung, dog. Five sections of lung are submitted for examination. At subgross magnification, the lungs are mildly atelectatic and alveolar septa are thickened and hypercellular. (HE, 8X)

quantities of fibrin (occasionally suspicious for capillary fibrin thrombi). Alveolar lumens contain increased numbers of free-floating macrophages, very rare neutrophils, and protein-rich fluid. Most intravascular erythrocytes contain small (1-3 micron) intracytoplasmic basophilic bodies, most visible in alveolar capillaries around the periphery of the histologic sections. Giemsa stains accentuate these small round faintly vacuolated organisms within erythrocytes.

Contributor's Morphologic Diagnoses:
Generalized histiocytic and neutrophilic alveolitis with multifocal capillary thrombosis and intravascular, intraerythrocytic parasites (Babesia vogeli)

Contributor's Comment:
Babesia spp. are tick-transmitted, apicomplexan, hemoprotozoan parasites. They are closely related to *Cytauxzoon spp*. and *Theileria spp*., and these three genera are termed piroplasmids. *Babesia spp*. was initially identified as a hemoparasite of cattle in Romania in 1888, causing hemoglobinuria, a syndrome termed "Red Water Fever". Soon after, similar organisms were identified in cattle in Texas, and today *Babesia spp*. are recognized worldwide as tick-borne parasites of wild and domestic ruminants, equids, pigs, dogs, and

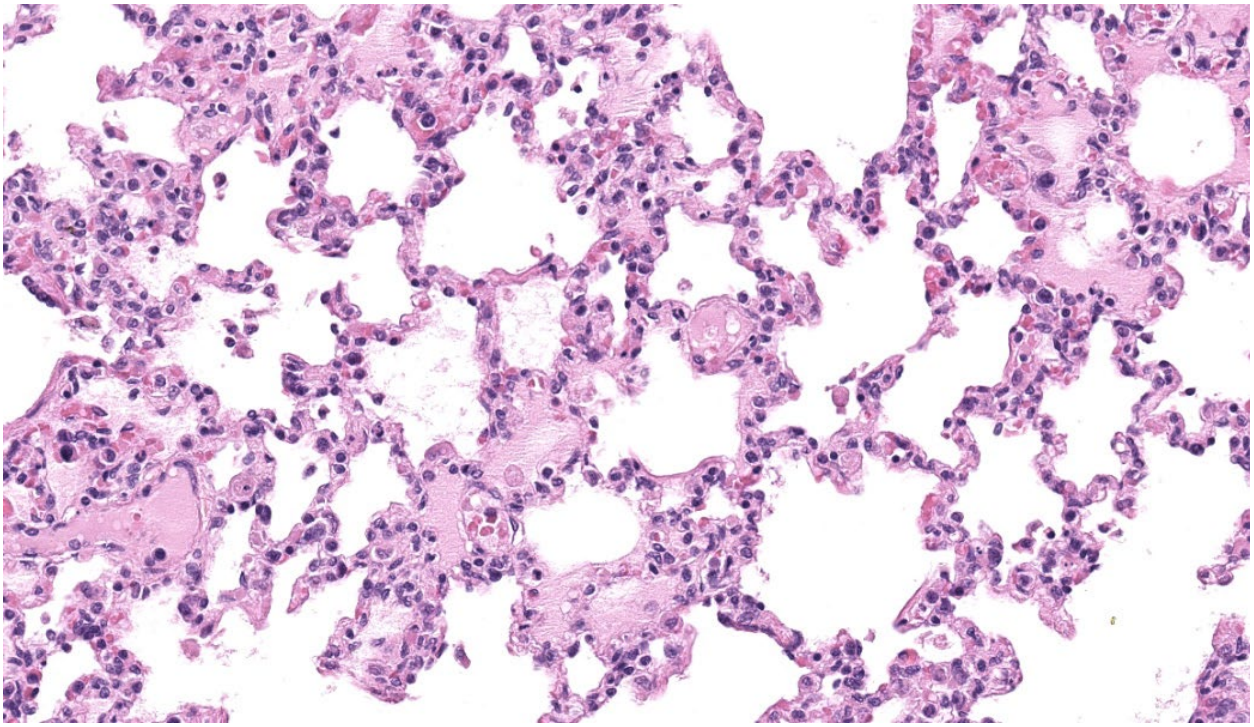

Figure 4-2: Lung, dog. Alveolar septa are markedly thickened by congestion, edema, and fibrin, and are hypercellular as a result of hypertrophy of septal macrophages, circulating neutrophils, and infiltration of the septa by additional macrophages and neutrophils. Alveoli contain edema fluid, protein, and foamy macrophages. (HE, 476X)

cats[8]. Internationally, these parasites are of economic concern due to their impact on livestock health and productivity. Humans are also susceptible to *Babesia spp*., including *B. microti, B. venatorum, B. divergens, B. duncani, B. divergens*, and other unnamed *Babesia spp.*, and human transmission has also been documented horizontally, or via blood transfusion[7].

Dogs have been reportedly infected with *B. vogeli, B. conradae, B. gibsoni, B. vitali, B. rossi, B. canis*, and unspeciated *Babesia* spp[7]. Original studies classified canine *Babesia spp*. based on morphology, with larger (3-5 micron) forms named *B. canis*, and smaller (1-3 micron) forms named *B. gibsoni*. However, it was suspected based on differences in pathogenicity that *B. canis* was heterogeneous, and with the advent of molecular diagnostic techniques, at least four small and four large *Babesia* parasites have been identified. *B. canis* was reclassified into three separate species (*B. canis, B. vogeli, B. rossi*)[5]. *Babesia vogeli* is transmitted by the Brown Dog tick (*Rhipicephalus sanguineus*) and has a wide endemic range throughout tropical and subtropical regions of the world, occasionally extending into cooler latitudes[5].

In dogs, clinical babesiosis varies from mild to severe forms, manifesting as hemolysis and secondary systemic hypoxic and inflammatory organ injury, organ dysfunction, shock, and death[1]. The severity of clinical disease varies with a number of factors, including parasite species, and host age, immune status, and the presence of concurrent infection. The least pathogenic species recognized is *B. vogeli*, the

most virulent is *B. rossi*. While *B. vogeli* more frequently subclinical, it is notably more pathogenic in puppies younger than 3-4 months[5]. A number of mechanisms have been implicated for the parasite-mediate hemolysis, including direct parasite-mediated injury, osmotic fragility of infected erythrocytes, oxidative stress, and secondary immune-mediated cell membrane injury, resulting in mixed intravascular and extravascular hemolysis[5].

Common hematologic abnormalities include anemia, thrombocytopenia, and variable leukopenia or leukocytosis. Serum biochemical abnormalities include increased activity of aspartate aminotransferase and alanine aminotransferase, hyperbilirubinemia, hypoalbuminemia, and variable electrolyte and acid-base imbalances. Eichenberger *et al.* reported that the prognosis of clinically ill dogs infected with *Babesia canis* was negatively impacted by hyperlactatemia, leukopenia, hyperphosphatemia, hypertriglyceridemia, and hypoproteinemia[1]. Similarly, prognosis for *B. rossi* infection has been found to be impacted by hyperlactatemia, hypoglycemia, clinically compromised circulation/consumptive coagulopathy, and increased serum cortisol[1].

Preliminary diagnosis of acute clinical Babesiosis typically requires evaluation of blood smears, usually using Giemsa or Wright's stain. The likelihood of diagnosis, particularly with large *Babesia* spp., may be improved through specific evaluation of blood smears from capillary beds (ear tips, toenail) or evaluation of cells beneath the buffy coat of a hematocrit tube. In chronic cases, parasitemia may be minor and intermittent, making blood smear diagnosis more challenging[5].

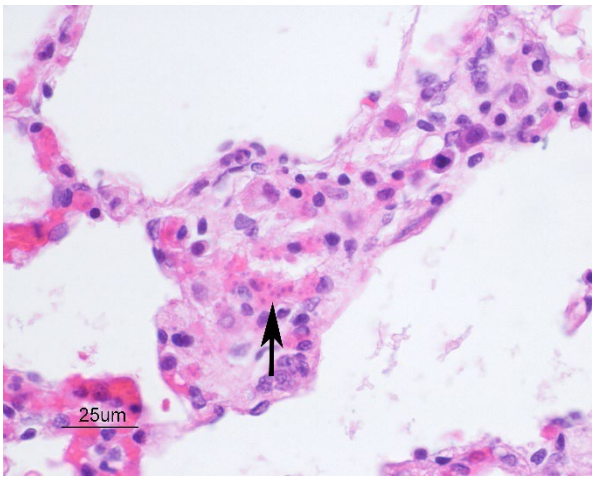

Figure 4-3: Lung, dog. Erythrocytes contain small (1-3 micron) intracytoplasmic basophilic bodies (arrow).(Photo courtesy of: Animal Health Laboratory, University of Guelph, http://www.guelphlabservices.com/AHL/) (HE, 600X)

PCR, immunofluorescence, and ELISA testing have also improved the rate of diagnosis of babesiosis, and PCR testing has become the principle means of diagnosis and species determination[5]. However, false negative results may be seen in cases of chronic babesiosis because of limitations similar those of blood smear diagnosis, and detection of chronic/carrier animals often requires repeated testing and use of complimentary testing methods[2,6].

Importation of domestic dogs to Canada from other countries is becoming more common, though this is not well-tracked and reliable statistics are not available. This pattern of importation provides a risk of infectious diseases being imported along with them, and because some of these pathogens depend on environments or vectors not found in Canada, recognition may be delayed. However, because of the presence of competent tick vectors in some regions of Canada, the importation of dogs with babesiosis may provide a risk for disease

transmission to local dog populations. Hemotropic parasites such as *Babesia, Anaplasma, Mycoplasma, Bartonella, Ehrlichia*, and *Rickettsia* spp. should be considered as possibly subclinical infections in imported dogs, and blood testing (including review of blood smears) prior to importation of dogs from endemic areas is recommended to reduce the risk of establishing infection within Canadian tick populations.

Contributing Institution:
Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada
http://www.guelphlabservices.com/AHL/

JPC Diagnoses:
Lung: Pneumonia, interstitial, histiocytic and neutrophilic, subacute, diffuse, moderate, with intraerythrocytic piroplasms.

JPC Comment:
MAJ Fiddes chose this case specifically because it was going to be a diagnostic challenge. The intraerythrocytic piroplasms of *Babesia sp.* are quite difficult to see until you find one and got an eye for them within erythrocytes; then, they were much easier to spot. A few participants were successful in finding these small parasite. Along with discussion on the basics and life cycle of *Babesia sp.*, which the contributor's comment beautifully covers, conference goers participated in a matching game of other boards-worthy infectious hemotropic agents of note. These agents and their matching facts included *Anaplasma sp.*, which may infect platelets, *Theileria sp.*, which infect leukocytes and are another genus of piroplasm, *Mycoplasma sp.*, which tend to reside in an epicellular location, and *Cytauxzoon felis,* yet another piroplasmid and one that has a bobcat reservoir host.

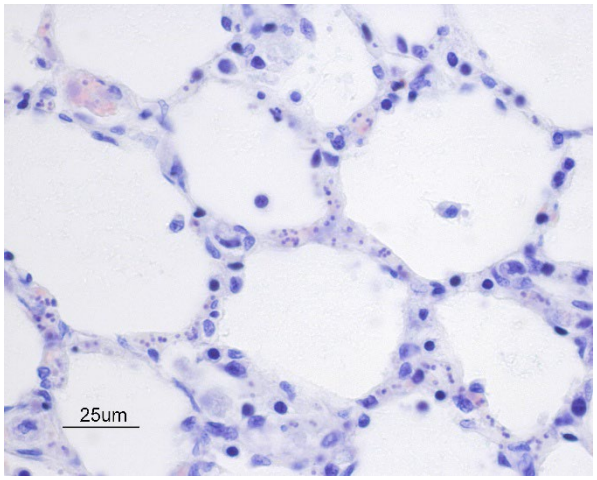

Figure 4-4: Lung, dog. A Giemsa stain helps delineate the punctate intracytoplasmic intraerythrocytic inclusions.(Photo courtesy of: Animal Health Laboratory, University of Guelph, http://www.guelphlabservices.com/AHL/) (Giemsa, 600X)

Babesia sp. are apicomplexan piroplasmid protozoans that infect erythrocytes in numerous species, ultimately lysing infected red blood cells (RBCs) and resulting in intravascular hemolysis within the host. As mentioned,

by the contributor, there are several species of *Babesia* that infect dogs, and there is a wide range of severity in the clinical manifestations amongst these. The focus here will primarily be on *Babesia canis,* which infects dogs, and *Babesia bovis*, the species responsible for most *Babesia* infections in cattle and has a substantial amount of research behind it.

Most of the virulence factors that *Babesia* is equipped with are responsible for survival and pathogenicity. Like other apicomplexans, *Babesia's* virulence factors are generally secreted to the host cell surface, where they structurally remodel and biochemically modify the infected RBC's ability to interact with specific host proteins in an effort to promote their own survivability. The most notable of

these numerous virulence factors is the Virulence, Evasion, and Secretion-associated (VESA) proteins. VESA is responsible for inhibiting the binding of infected erythrocytes to endothelial cells, as well as reversing the binding of already-adhered erythrocytes.[3,7] This ensures that, when the piroplasms have reproduced via binary fission and are ready to lyse the infected red cell, that they will remain within the vasculature to be picked up by a tick vector.

VESA proteins cluster on the surface of erythrocyte within ridges that are structurally induced by *Babesia*.[3] The number of ridges increases as the piroplasmids grow and multiply within the erythrocyte.[3] With VESA being expressed on the surface of infected red cells, the immune system should be able to locate and target those cells for antibody-mediated immune response, right? Yes, but no. *Babesia bovis* has demonstrated that it can express antigenic variants of VESA through epigenetic alterations and/or genomic recombination at the locus of active transcription that is responsible for the production of VESA proteins.[3,7] This alters the VESA protein enough antigenically to become a constant moving target for immune cells, contributing to the chronic and recurrent nature of babesiosis.[3] It is yet another prime example of the evolutionary arms race between infectious agents and immune cells, and it is currently speculated that many other virulent *Babesia sp* can perform the same epigenetic alterations to their VESA antigens.

There was some discussion on the presence of megakaryocytes within the affected lung sections in this case. Megakaryocytes can be present normally in low numbers in the lungs or can be seen in increased numbers secondary to an immune response. Megakaryocytes normally function to produce platelets, and a single megakaryocyte can make them by the thousands. In an immune response, though, megakaryocytes display an impressive range of functions related to pathogen recognition, antigen presentation, and inflammatory modulation.[4] Lung megakaryocytes, specifically, express higher levels of pathogen recognition receptors (PRRs), specifically, toll-like receptors (TLRs) and C-type lectin-like receptor (CLEC). Lung megakaryocytes also sense and uptake pathogens, can release a gamut of inflammatory cytokines, function as antigen-presenting cells, and participate in immune responses against pathogen invasion.[4] Because of their wide range of functions and key physiologic roles, megakaryocytes are sometimes referred to as an "omnipotent" cell type.[4] In this case, it was the opinion of conference participants that the megakaryocytes were likely present due to the inflammation and protozoal infection due to the higher-than-expected number seen within section.

References:

1. Eichenberger RM, Riond B, Willi B, Hofmann-Lehman R, Deplazes P. Prognostic markers in acute Babesia canis infections. J Vet Intern Med. 2016;30:174-182.
2. Goo Y, Jia H, Aboge GO, et al.. Babesia gibsoni: Serodiagnosis of infection in dogs by an enzyme-linked immunosorbent assay with recombinant BgTRAP. *Exp Parasitol.* 2008;118:555-560.
3. Hakimi H, Yamagishi J, Sakaguchi M, Fathi A, Lee JS, Verocai GG, Kawazu SI, Asada M. ves1α genes expression is the major determinant of Babesia bovis-infected erythrocytes cytoadhesion to endothelial cells. *PLoS Pathog*. 2025; 21(4):e1012583.

4. Hua T, Yao F, Wang H, Liu W, Zhu X, Yao Y. Megakaryocyte in sepsis: the trinity of coagulation, inflammation and immunity. *Crit Care.* 2024;28(1):442.
5. Irwin PJ. Canine babesiosis: from molecular taxonomy to control. *Parasit Vectors*. 2009;2(suppl 1):1-9.
6. Jefferies R, Ryan UM, Jardine J, et al. Babesia gibsoni: Detection during experimental infections and after combined atovaquone and azithromycin therapy. *Exp Parasitol.* 2007;117:115-123.
7. Schetters T. Mechanisms Involved in the Persistence of *Babesia canis* Infection in Dogs. *Pathogens*. 2019;8(3):94.
8. Schnittger L, Rodriguez AE, Florin-Christensen M, Morrison DA. Babesia: A world emerging. *Infect. Genet Evol*. 2012;12:1788-1809.
9. Uilenberg G. *Babesia* – A historical perspective. *Vet Parasitol*. 2006;138:3-10.