Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #12 06 December 2023

CASE I:

Signalment:
12-year-old male neutered domestic shorthair cat (*Felis catus*)

History:
The cat was rescued from a hoarding situation in the year prior to euthanasia. The cat was evaluated for weight loss, hyporexia, icterus, diarrhea, polyuria, polydipsia, hypertension, neurologic signs (circling, head tilt, nystagmus, dullness), eyelid and ear canal masses, retinal detachment, anterior uveitis, and blindness. Lymphoma was suspected and the cat was euthanized.

Gross Pathology:
At necropsy the cat was icteric and the liver, spleen, intestinal walls, and mesenteric lymph nodes were enlarged. The capsular surface of the kidneys was pitted (chronic interstitial nephritis). As noted clinically, there were 4-8 mm raised cutaneous, variably ulcerated or alopecic nodules primarily concentrated around the face, paws and pinnae. There was bilateral axial corneal opacity and roughening (ulceration and edema). In the left eye, the anterior chamber contained flocculent white to yellow material (fibrin) and the iris was diffusely expanded by tan material. In both eyes, the retina and choroid were variably expanded by tan material.

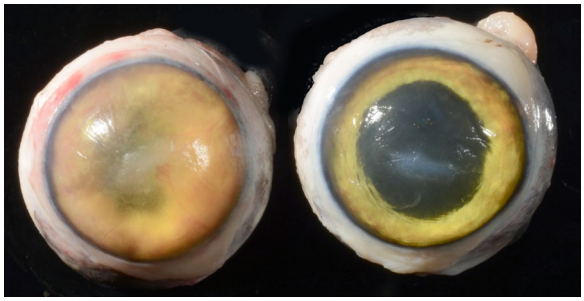

Figure 1-1. Eye, cat. There was bilateral axial corneal opacity and roughening (ulceration and edema). (*Photo courtesy of:* University of Tennessee, College of Veterinary Medicine, Department of Biomedical and Diagnostic Sciences, http://www.vet.utk.edu/departments/path/index.php)

Microscopic Description:
The left eye was more severely affected, but the following lesions are apparent in both eyes. The iris, ciliary body, trabecular meshwork, choroid and retina are variably expanded by sheets of large foamy macrophages that contain myriad, round to oval, 2-4 µm, basophilic yeast surrounded by a clear halo. There are associated lymphocytes and plasma cells. The retina is detached and there is loss of photoreceptor outer segments. There is thinning and mixing of all retinal layers (atrophy). Retinal blood vessels are often cuffed by lymphocytes (retinitis). In some sections, the subretinal space contains abundant fibrin with fewer lymphocytes and yeast-laden macrophages. The lens or lens

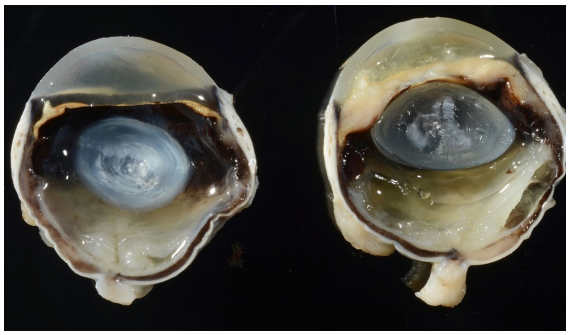

Figure 1-2. Eye, cat. In the left eye, the anterior chamber contained flocculent white to yellow material (fibrin) and the iris was diffusely expanded by tan material (*Photo courtesy of:* University of Tennessee, College of Veterinary Medicine, Department of Biomedical and Diagnostic Sciences)

capsule is present in some sections and exhibits mild peripheral lens fiber swelling and posterior migration of lens epithelial cells (cataract). There is mid-stromal and deep corneal vascularization. In some sections, there is axial corneal ulceration with associated bacterial colonies, fibrin and neutrophils; the adjacent intact corneal epithelium is attenuated.

Contributor's Morphologic Diagnosis:
Eye: Severe diffuse granulomatous endophthalmitis with intrahistiocytic yeasts, retinal detachment, cataract and corneal ulceration.

Contributor's Comment:
The morphology of the yeasts is most consistent with *Histoplasma* spp. At necropsy, gross lesions were also identified in the skin, liver, spleen, intestines and lymph nodes, and microscopic examination identified similar lesions and yeast in those tissues as well as in the kidneys and lungs.

In the United States, histoplasmosis is usually due to *Histoplasma capsulatum* and cases occur most commonly in midwestern and southern states. Bird and bat feces are a common source of infection. *Histoplasma* spp. are dimorphic fungi and infection is usually acquired by inhalation of microconidia. In the lungs, the microconidia take on the yeast form. The yeast are phagocytized and proliferate by budding intracellularly. The yeast can then spread via the blood and lymphatics to distant sites resulting in systemic infection. Although the lungs are usually the primary site of infection, the skin and gastrointestinal tracts may also be primary sites of infection.

As with many fungal infections, immunocompromised individuals are more susceptible to infection and dissemination. In some immunocompetent hosts, *Histoplasma* spp. can be dormant until reactivation during times of immunosuppression.[1]

Clinical signs and lesions depend on the organs affected. As in this case, the lungs, liver, spleen and lymph nodes are commonly affected in systemic histoplasmosis.[1]

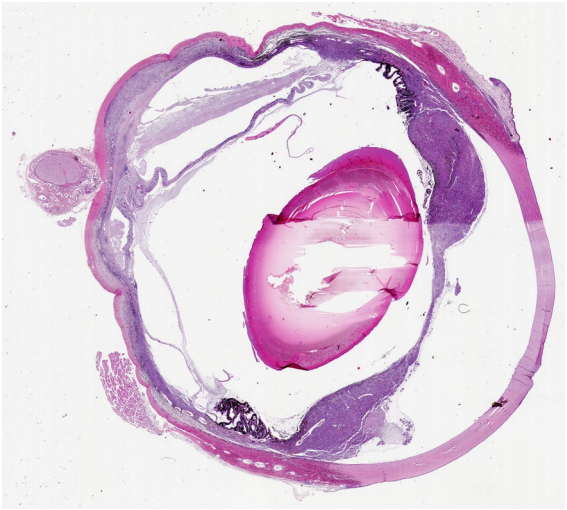

Figure 1-3. Eye, cat. The uvea and choroid are diffusely and markedly expanded by a cellular exudate which extends into the anterior and posterior chambers. There is retinal detachment and atrophy with subretinal exudate. (HE, 5X) (*Photo courtesy of:* University of Tennessee, College of Veterinary Medicine, Department of Biomedical and Diagnostic Sciences)

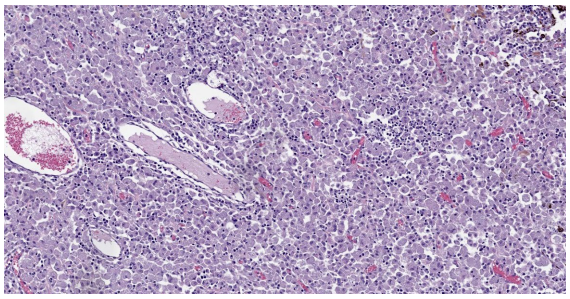

Figure 1-4. Iris, cat. The iris is expanded by sheets of large macrophages with fewer lymphocytes. (HE, 5X)

Ocular involvement may be present in about 24% of cats and may include blepharitis, conjunctivitis, chorioretinitis, panuveitis, panophthalmitis, retinal detachment, and optic neuritis.[1,2]

Contributing Institution:
University of Tennessee
College of Veterinary Medicine
Department of Biomedical and Diagnostic Sciences, http://www.vet.utk.edu/departments/path/index.php

JPC Diagnosis:
Eye: Endophthalmitis, granulomatous, diffuse, severe, with retinal detachment and atrophy, and numerous intrahistiocytic yeasts.

JPC Comment:
Histoplasma capsulatum is found primarily in soils enriched in bird and bat guano where it exists as a conidia-forming mold. Once inhaled, the pleasant 37°C temperature of the host converts the organism to a yeast phase composed of 2 to 4µm oval budding yeasts that can be found both extracellularly and within macrophages.[3] There are three varieties of *Histoplasma capsulatum* of veterinary importance: *Histoplasma capsulatum* var. *capsulatum,* the most common variant and the subject of this comment; *Histoplasma capsulatum* var. *duboisii*, which primarily causes cutaneous disease in humans and baboons in Africa; and *Histoplasma capsulatum* var. *farciminosum*, which causes epizootic lymphangitis, or "pseudoglanders," in equids.

Exposure to *Histoplasma* spp. in endemic areas is common, but disease is rare. As the contributor notes, disease, when present, primarily affects the lungs; systemic disease typically results only when the exposed host lacks sufficient cell-mediated immunity to arrest the macrophage-associated spread of yeast throughout the body. Localized, pulmonary, and disseminated histoplasmosis have been described in both domestic and wild felids, and histoplasmosis is reported to be the second most common systemic fungal infection in cats (second only to cryptococcosis).[5] The most common body systems affected are the respiratory tract, eyes, musculoskeletal system, hemolymphatic organs, and the skin.[5]

It is unclear how commonly feline histoplasmosis affects the eyes. In one case series, all cats with confirmed disseminated histoplasmosis had ocular lesions discovered on ophthalmic evaluation, while more recent reports place this number at 24%.[2,5] Typical ocular signs include optic neuritis, anterior uveitis, panuveitis, endophthalmitis, choroiditis/chorioretinitis, often with retinal detachment, and secondary glaucoma.[5]

Research in humans and in animal models has shown that *H. capsulatum* organisms can persist in tissues in a dormant state for many years.[5] In immune-competent animals, the immune system retards or eliminates the organisms' ability to replicate but does not kill them, leading to the granulomatous lesions

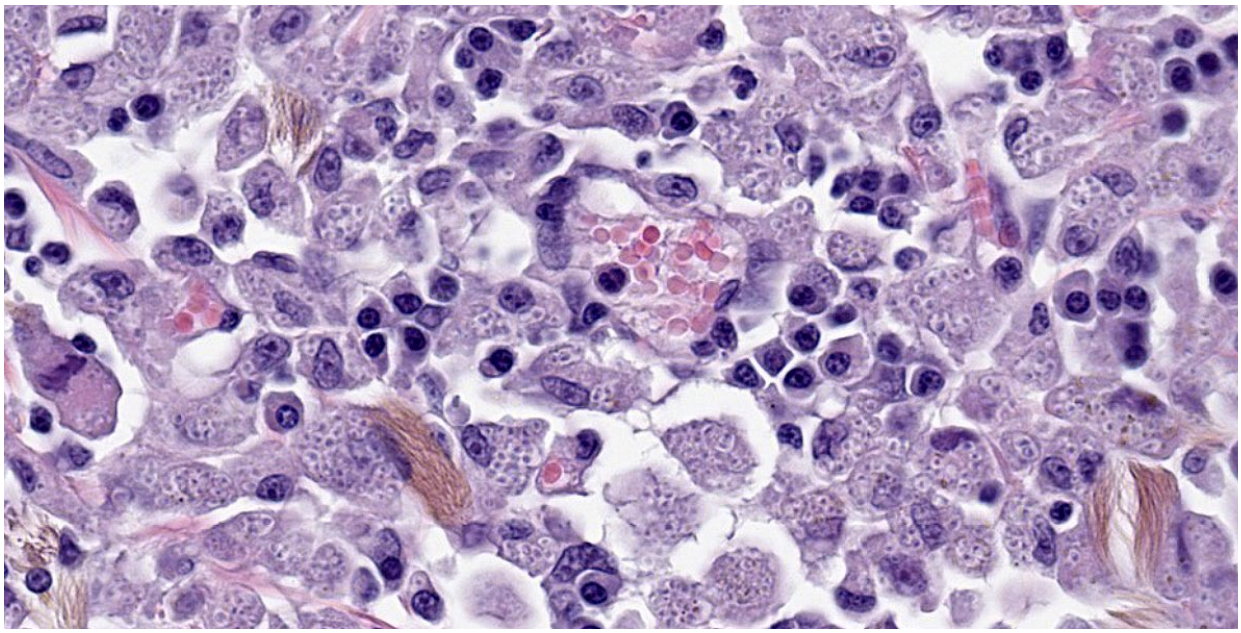

Figure 1-5. Eye, cat. Macrophages contain numerous intracytoplasmic 2-4um round to elliptical yeasts. Interspersed melanocytes contain golden brown filamentous pigment characteristic of the feline eye. (HE, 783X)

that are characteristic of the disease.[5] Reactivation of disease may occur with immune suppression, with disseminated disease occurring many years after the host has been removed from exposure to the organism.[5] This has implications for ocular histoplasmosis as the eye is an immunologically sequestered tissue that is separated from the rest of the body by the blood-aqueous and blood-retinal barriers.[5] While sequestration of *H. capsulatum* in the eye and subsequent systemic dissemination after discontinuation of antifungal therapy has not been definitively proven, studies of ocular *Blastomyces* spp. infections suggest that fungal persistence in the eye can delay clearance of the organism and perhaps serve as a reservoir for recrudescent infection.[5] The ability to effectively treat ocular histoplasmosis is further complicated by the poor sensitivity of current laboratory tests, such as serial urine and serum antigen assays, at detecting histoplasmosis that is restricted to ocular tissues.[5]

Detecting the organism was not a particular challenge in this case, however, which delighted conference participants with its somewhat ostentatious display of yeast-laden macrophages. The florid nature of the infection frustrated resident attempts to pinpoint exactly which ocular structures were involved. Our moderator for the week, Dr. Rachel Neto, Assistant Clinical Professor at Auburn University College of Veterinary Medicine and ocular pathology enthusiast, allowed that there might be scattered inflammation within the sclera, but felt that this was primarily a uveocentric disease process and preferred to describe the lesion as an endophthalmitis rather than a panophthalmitis.

A considerable amount of discussion centered on the need to read through processing and sectioning artifacts. Dr. Neto particularly noted that she would be cautious in interpreting changes to the drainage angle based on the examined section alone as the section did

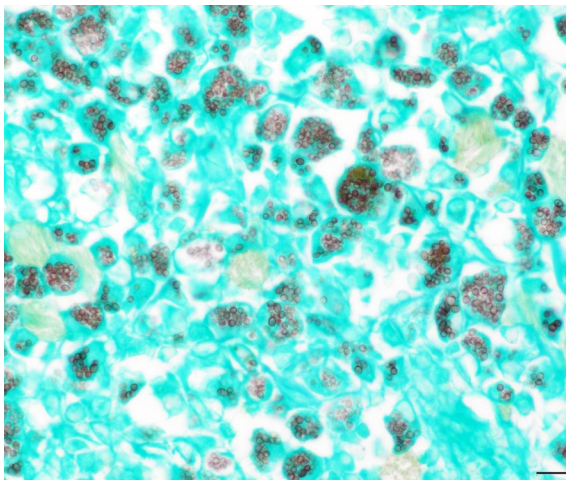

Figure 1-6. Eye, cat. A GMS stain demonstrates the yeasts. (GMS, 400X) (*Photo courtesy of:* University of Tennessee, College of Veterinary Medicine, Department of Biomedical and Diagnostic Sciences)

not include the pupil and was likely a parasagittal cut.Neoplasia should be included on any differential list that is based solely on gross appearance. As this is a cat (evidenced here by the many melanocytes containing filamentous, golden pheomelanin), feline diffuse iris melanoma, lymphoma, and post-traumatic sarcoma should be on the list. Infectious differentials include FIP, *Toxoplasma,* and the dimorphic fungi. On histologic exam, however, the size, morphology, and intrahistiocytic location of the organism narrows the list considerably to *Histoplasma* and *Leishmania.* Dr. Neto also discussed a dimorphic fungus, *Blastomyces helicus*, that looks virtually identical to *Histoplasma capsulatum*, is a recently-described cause of feline pneumonitis, and should be considered when confronted with *Histoplasma*-like organisms in cats.[4]

References:

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CASE II:

Signalment:

3-day-old male White Shorthorn calf *(Bos taurus)*

Figure 2-1. Eyes, right and left, calf. Both globes are smaller than normal, with the right globe (on the left in this image) being considerably smaller than the contralateral eye. (*Photo courtesy of:* Department of Pathobiology and Population Sciences (PPS), Royal Veterinary College, Hawkshead Lane, Brookmans Park, Hatfield, Hertfordshire, AL9 7TA. www.rvc.ac.uk)

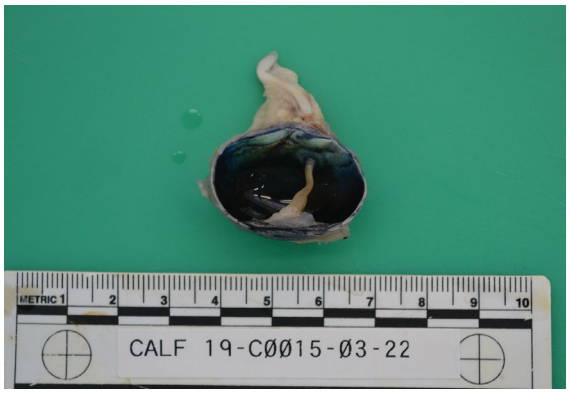

Figure 2-2. Eye, right. The retina is not anchored and attaches to th posterior asprect of a hypoplastic lens. (*Photo courtesy of:* Department of Pathobiology and Population Sciences (PPS), Royal Veterinary College, Hawkshead Lane, Brookmans Park, Hatfield, Hertfordshire, AL9 7TA.)

History:
A male, White Shorthorn calf was delivered with assistance and born blind. This was the third calf to be born blind in the last two calving seasons. All blind calves were sired by the same White Shorthorn bull and offspring from other sires were unaffected.

Initially, the submitting veterinary surgeon suspected Vitamin A deficiency during gestation. However, a bolus strategy containing Vitamin A had no effect. Also, cohort sampling of 10 cows revealed unremarkable vitamin A levels in 9/10 cows and, therefore, the herd relevance of Vitamin A deficiency was unclear. The herd is vaccinated against BVD and all animals have tested negative for BVD.

Clinical examination of the blind calf by veterinary ophthalmologists revealed bilaterally microphthalmic globes, a wandering nystagmus, and a tendency for ventral rotation. Both pupils were small and the pupillary light reflexes and dazzle reflexes were absent. Close examination of both eyes revealed anterior segment dysgenesis with microcornea, shallow anterior chambers and small pupils. Detailed assessment of the lenses was not feasible due to small pupil size. The calf was euthanized by barbiturate injection.

Gross Pathology:
The ocular globes are mildly sunken into the orbits. Both globes are reduced in diameter, measuring 2.7 x 2.5 cm on the right (OD) and 3.1 x 2.7 cm on the left (OS), respectively. Bilaterally, there are multifocal scleral haemorrhages. Examination of the cut-sections post-fixation reveals both eyes have little obvious lens remaining. The left eye shows collapse of the anterior chamber and there are presumptive lens remnants. These are connected by a cream colored, soft membrane to the posterior pole of the eye. The other organs, including the cerebrum, cerebellum and brainstem were grossly unremarkable.

Laboratory Results:
BVDV PCR (spleen): Negative.

Microscopic Description:
As both eyes showed similar macroscopic and histopathological changes, only the left globe is described below.

Eye (OS): Compressing against the posterior aspect of the ciliary body and the posterior pole of the lens and displacing these structures anteriorly, there is a diffusely detached, disorganized and folded retina, which crosses the posterior chamber and joins with the optic nerve head. The underlying retinal pigmented epithelium is multifocally rounded and hypertrophied (tombstoning).

The dysplastic retina is characterized by the following histological features: multifocally the outer nuclear layer forms numerous tubular structures and rosettes surrounding a central space containing eosinophilic fibrils (presumptive necrotic rods and cones) admixed

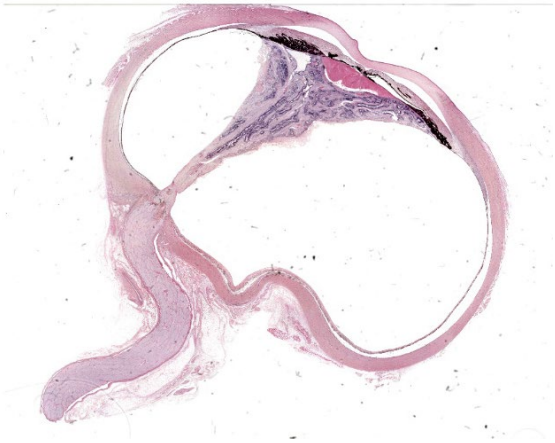

Figure 2-3. Eye, right. A cross section of the globe demonstrates the lack of retinal anchoring, marked disorganization of retinal layers, and attachment to the posterior surface of a lens that is flattened craniocaudally, and collapse of the anterior chamber. (HE, 5X)

with scattered degenerate neutrophils. The outer plexiform layer surrounding these rosettes and tubules is diffusely thinned and the inner and outer nuclear layers frequently coalesce. The inner plexiform layer, ganglion cell layer, nerve fiber layer and internal limiting membrane multifocally frequently blend together and are not readily distinguishable. However, the ganglion cell layer can be occasionally distinguished in areas of retinal folding. Multifocally, randomly distributed throughout the dysplastic retina there are large lakes of amorphous eosinophilic material admixed with melanocytes and karyorrhectic cellular debris (lytic necrosis). The nerve fibers within the adjacent optic nerve head are mildly vacuolated and there is a diffusely, mildly increased cellularity (likely oligodendrocytes) within the endoneurium of the optic nerve. As a result of the aforementioned changes, the anterior chamber is markedly collapsed, and the iris and ciliary body attach to the anterior pole of the lens (posterior synechiae).

The lens shows one or more of the following histological features: markedly reduced size, liquefaction of the cortical and nuclear fibers and moderate numbers of swollen cells with scattered retained nuclei (bladder cells). The posterior contour of the lens is uneven and there is rupture of the posterior capsule with formation of multifocal Morgagnian globules (cataract). Fragments of ruptured lens stroma are frequently surrounded by the previously described dysplastic retina. The corneal stroma is multifocally mildly edematous.

Contributor's Morphologic Diagnosis:
Globe (OS): Severe retinal dysplasia with posterior synechiae and secondary cataract.

Contributor's Comment:
Retinal dysplasia is an anomaly of the neuroectoderm that can be defined as abnormal differentiation of the retina characterized by glial proliferation and disorganization of the retinal layers.[11] As ruminants are born with mature retinas, unlike dogs and cats which continue to have post-partum retinal development, the underlying etiological cause for the retinal dysplasia in ruminants occurs in utero. In cattle and ruminants, common etiological causes include: viral infection (e.g., BVDV or bluetongue virus), hypovitaminosis A, and genetic causes such as those documented in Shorthorn calves.[1,3,6,10,11]

In cattle, post-necrotic retinal scarring of the developing retina associated with infection by BVDV between 79 and 150 days of gestation is the most frequently studied retinal dysplasia of viral aetiology.[11] The most significant findings suggesting a viral versus a genetic cause are the presence of inflammatory infiltrates, post-necrotic retinal scarring, and scarring within the optic nerve and the choroid. BVDV has an affinity for neural tissues and, therefore, all calves with retinal dyspla-

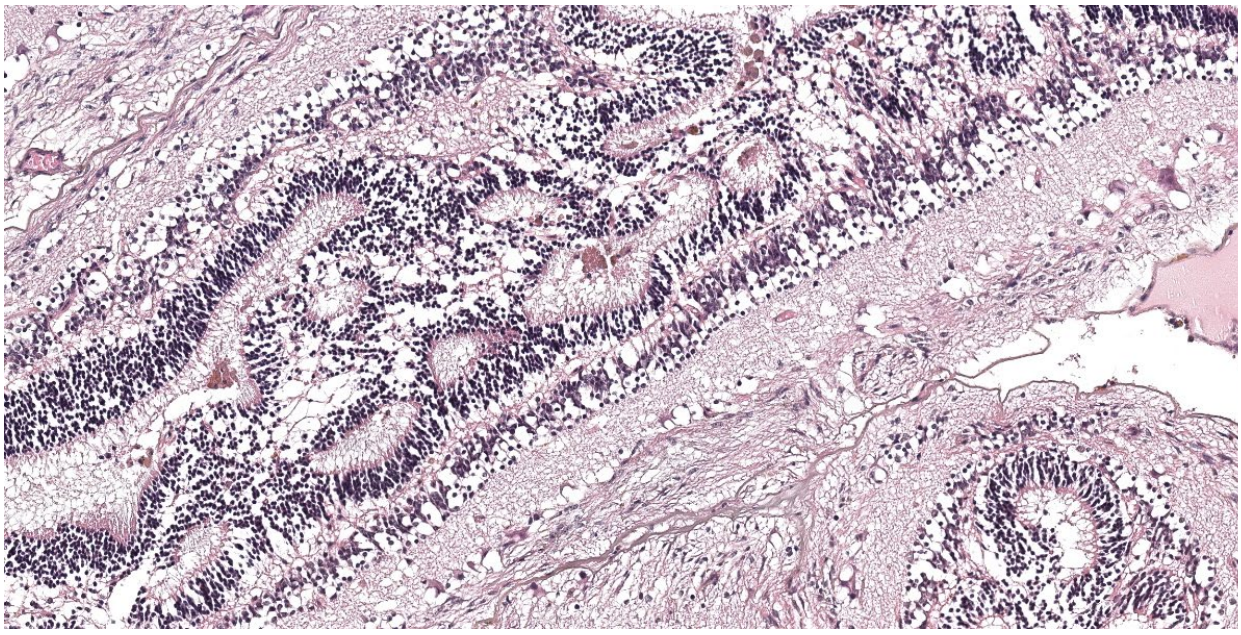

Figure 2-4. Right eye, retina. There is marked disorganization of the retina with folding upon itself. There is blending of the inner and outer nuclear layers. Rosettes formed from these combined layers, with photoreceptors projecting into the lumens of the rosettes, are common. (HE, 159X)

sia induced by the virus should also have cerebellar hypoplasia with or without hydrocephalus or hydranencephaly. While areas of necrosis are seen within the retina in this case, there was no evidence of retinal scarring or cerebellar hypoplasia, hydrocephalus, hydranencephaly or inflammation and the calf had a negative result from a BVDV PCR, making this differential less likely.

Bluetongue virus has only been described in the literature as causing retinal dysplasia in sheep, and the United Kingdom is currently free from the disease, with the last outbreak occurring in 2007.[1]

Retinal dysplasia has been described in some cases of hypovitaminosis A. This deficiency has been linked to feeding of brewer's grain, sorghum, or wheat straw which all contain low β-carotene activity and low potential vitamin A activity.[10] However, in cases of hypovitaminosis A there are usually additional changes, including a reduction in the size of the optic nerve, which were not observed in this case. Furthermore, the clinical history in this case of incidences being limited to calves from one sire and the non-responsiveness to Vitamin A supplementation the following calving season make involvement of hypovitaminosis A very unlikely.

To conclude, the most likely cause of the retinal dysplasia in this calf is a 'true' retinal dysplasia with an underlying genetic etiology, especially as in this case only calves from the same sire showed similar ocular changes. The previously documented cases of retinal dysplasia in Shorthorn cattle demonstrated a concurrent hereditary internal hydrocephalus which was not grossly observed in the submitted case. A recessive or incompletely penetrant dominant mode of inheritance was suggested, but the exact genetic mutation in Shorthorn cattle has not been identified.[3] Similarly, a genetic retinal dysplasia in Hereford calves has been described with a proposed dominant inheritance

of varied expressivity although again, the underlying genetic mutation has not been defined.[5] Finally, other breeds of cattle that have suspected congenital retinal dysplasias documented in the literature include Simmentals and Japanese Black Cattle.[8,9]

The pathogenesis of retinal dysplasia in cattle is not as well characterized as in other domestic species, such as dogs, but is expected to follow similar mechanisms which are described below.[11]

Primary (type 1) or true retinal dysplasia is rare. It occurs due to failure of maturation by an inherently defective retinal pigmented epithelium (RPE) or failure of apposition of the two layers of the optic cup. The condition is histologically characterized by retinal rosettes, retinal folds, blending of the nuclear layers, glial scars, loss of retinal cells and retinal detachment. Rosettes should be composed of a central lumen encompassed by 1-3 layers of neuroblasts.

Secondary (type 2), or post-necrotic retinal dysplasia, is common in cattle. Secondary retinal dysplasia occurs when an infectious cause (most likely viral-induced) triggers an initial nonsuppurative panuveitis and retinitis with an associated multifocal retinal and choroidal necrosis. Over several weeks the inflammation reduces and there are scant remnants of inflammatory cells remaining microscopically and only post-necrotic scarring. The condition is characterized histologically by the presence of residual inflammation and post-necrotic retinal scarring, optic nerve scarring, and choroidal scarring. The RPE undergoes reactive hyperplasia, migration into the scarred retina or forms metaplastic, multilayered fibroglial plaques to replace the simple cuboidal epithelium. The nuclear layers are disorganized and there is rosette formation. Lesions are more common bilaterally in the non-tapetal retina and are often asymmetrical.

Retinal folding is a type of retinal dysplasia that is common in dogs and is caused by an unbalanced growth rate between the retina and the outer layers of the optic cup (choroid and sclera) or defective RPE signaling. Histologic examination reveals infolding of the neuroblastic layer away from the RPE. The folds may be transient and disappear with continued choroidal and scleral growth.

Species	Cause
Sheep	Bluetongue virus
Dogs	Adenovirus, herpesvirus, genetically inherited breed-related dysplasia (English Springer Spaniel, Collies, Miniature Schnauzers, Labrador Retrievers, Samoyeds, Dobermans, Akitas, Chow Chows, Australian Shepherd dogs).[7]
Cats	Feline leukemia virus, feline panleukopenia virus, genetically inherited breed-related dysplasia (Abyssinian).
Chickens	A genetic retinal dysplasia linked to a mutation in the *MPDZ* gene has been identified.[4]

Table 1-1: Selected causes of retinal dysplasia.

Contributing Institution:
Department of Pathobiology and Population Sciences (PPS)
Royal Veterinary College
Brookmans Park, Hatfield, Hertfordshire
www.rvc.ac.uk

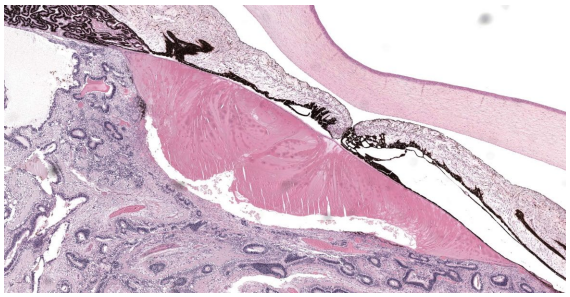

Figure 2-5. Right eye, retina and lens. The dysplastic retina is attached to the posterior aspect of the lens (there is artifactual separation due to processing). There is no capsule at the area of attachment. The lens is flattened in a craniocaudal direction. There is collapse of the anterior chamber. (HE, 32X)

JPC Diagnosis:
Eye: Ocular dysgenesis with retinal dysplasia, retinolenticular adhesion, and cataract.

JPC Comment:
The contributor gives an excellent overview of the pathogenesis, classification, and histopathologic lesions of retinal dysplasia. The various manifestations of retinal dysplasia can best be understood in the context of ocular organogenesis, which begins with bilateral evaginations of the forebrain that become separated from the developing diencephalon by the optic stalks.[11] These evaginations, called optic vesicles, grow outward toward the surface ectoderm, where signaling from a thickened area of surface ectoderm called the lens placode causes the distal optic vesicles to evaginate. These evaginations form the bilayered optic cups, and signaling from and to the lens placode causes its evagination and separation from the ectoderm to form the developing lens.[12] The inner layer of the optic cup proliferates and becomes the retinal neuroepithelium, while the outer layer of the optic cup, via interactions with the surrounding mesenchyme, becomes the retinal pigmented epithelium (RPE). The surrounding mesenchyme subsequently develops into the vascular and fibrous tunics of the eye.[11,12]

The RPE is distinguished from the neural retina by the presence of pigment and by a monolayer epithelial arrangement as opposed to the pseudostratified epithelial arrangement of the neural retina. If these features are not present, this is considered a failure of RPE differentiation, which has implications for further ocular development as the RPE is required for subsequent retinal and ocular morphogenesis.[13] In early development, the neural retina and the RPE depend on intercellular cross-talk for normal development, and hyperplasia of one layer can occur at the expense of the other if this complex signaling is perturbed.[13]

As noted by the contributor, true retinal dysplasia is caused either by improper apposition of the two layers of the optic cup, leading to improper cell-cell signaling interactions during development, or to an improperly formed or defective RPE.[11] The histologic hallmark of true retinal dysplasia is the rosette, several most excellent examples of which are present in the examined slide, composed of a central lumen surrounded by 1-3 layers of neuroblasts with varying amounts of retinal differentiation and pink fibrils, representing photoreceptors, projecting into the lumen.[11]

In dogs, true retinal dysplasia occurs in combination with chondrodysplasia in several dog breeds, including the Labrador Retriever and Samoyeds, and retinal dysplasia may be accompanied by persistent hyaloid membranes and cataracts in these breeds.[11]

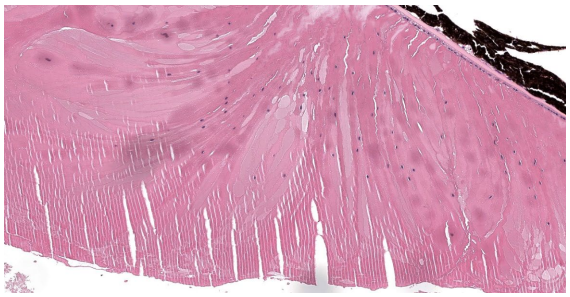

Figure 2-6. Right eye, lens. Lenticular changes include disorganization of posterior lens epithelium and cataract.

Conference participants quickly found themselves engaged in discussions at once semantic and existential. Several participants interpreted the lenticular changes and the attendant inflammation as phacoclastic uveitis; however, Dr. Neto felt the term inappapropriate given that it implies a lens capsule rupture. In this case, the lens, including the lens capsule, likely formed neither correctly nor completely. Can that which was never formed be ruptured? Similarly, the term "retinal detachment" implies that the retina was once attached to the RPE, perhaps an erroneous assumption in the context of retinal dysplasia, where the layers of the optic cup were likely never properly opposed. Can that which was never together be separated?

More practically, Dr. Neto noted that this case is a great example of why one should always evaluate the slide grossly prior to histologic examination. Doing so in this case reveals an entire calf eye that fits on a standard slide – a likely indication that the eye is too small. Dr. Neto then discussed the difference between nanophthalmos, where the eye is smaller than the control eye, but is anatomically intact, and microphthalmos. Microphthalmos comes in two varieties. In simple microphthalmos, the eye is small due to a developmental problem with the optic vesicle or due to difficulty maintaining intraocular pressures, but the eye is anatomically intact. Some consider simple microphthalmos and nanophthalmos to be equivalent conditions. Complex microphthalmos occurs when, as in this case, the eye is small but is anatomically abnormal, often with disorganized tunics. Microphthalmos can encompass anterior segment abnormalities such as cataract, as well as posterior segment abnormalities such as chorioretinal coloboma and retinal dysplasia.

Discussion of the morphologic diagnosis reflected the philosophical discussion noted above, with participants choosing to describe the retinal changes as simple dysplasia versus detachment and dysplasia. Some participants felt strongly that the chronic inflammation surrounding the lens should be included in the diagnosis, but the majority felt the inflammation was mild and relatively insignificant compared to the severe anatomic disorder present in this remarkable eye.

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CASE III:

Signalment:
Juvenile intact female Black Angus calf (*Bos taurus*)

History:
Seven weaned calves had a history of rapidly progressive neurologic signs, blindness, recumbency, and death. No obvious ocular lesions were appreciated clinically, and animals were unresponsive to dexamethasone and thiamine administration.

Gross Pathology:
A 200 kg calf had mild postmortem autolysis. The anterior chambers of the right and left eyes contained a moderate amount of fibrin and opaque fluid (hypopyon). Within the cerebral white matter, there was a focally extensive area characterized by numerous, up to 2 mm diameter, dark red to brown foci (infarcts). Additional gross lesions included a severe mural and valvular endocarditis of the right atrium and ventricle, fibrinosuppurative polyarthritis, suppurative bronchopneumonia, and fibrinosuppurative bilateral otitis media.

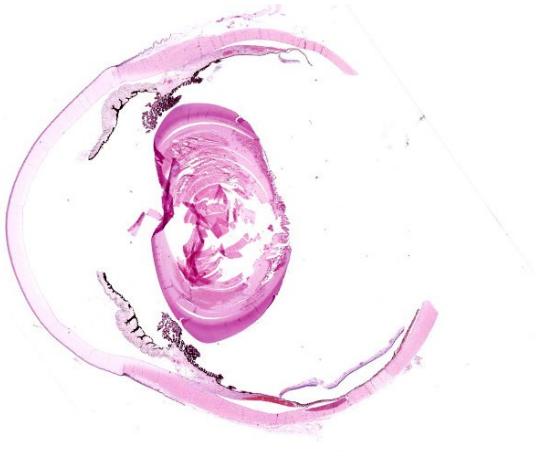

Figure 3-1. Eye, calf. A hemisection of the globe is submitted for examination. The caudal aspect of the globe, including the optic nerve, is not evident. (HE, 5X)

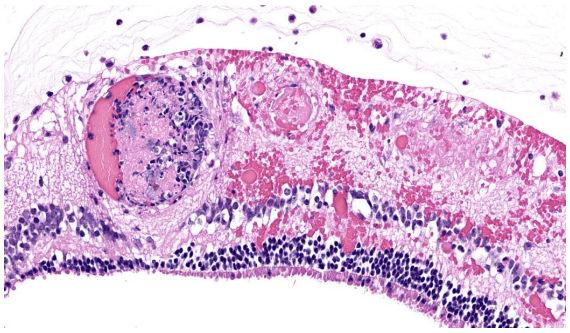

Figure 3-2. Retina, calf. There is vasculitis of small and medium-caliber vessels within the retina. Hemorrhage and edema distort the normal layered retinal architecture and result in a generalized spongiosis of the nerve fiber layers. (HE, 391X)

Laboratory Results:
Aerobic culture of synovial fluid, brain, and meningeal swab: No growth detected.
Mycoplasma bovis PCR, lung: Positive.
Mycoplasma bovis PCR, synovial fluid: Negative.
Histophilus somni PCR, brain: Positive.
Histophilus somni PCR, lung: Positive.

Microscopic Description:
Right eye: Frequently, small and medium-caliber blood vessels within the retina, choroid, anterior uvea, and optic nerve (not present in the section) are expanded with partial to complete occlusion of vascular lumina by fibrin thrombi containing small numbers of degenerate neutrophils and small numbers of gram-negative coccobacilli. The walls of affected blood vessels are transmurally infiltrated and effaced by moderate numbers of viable and degenerate neutrophils and small amounts of fragmented cellular and nuclear debris (necrotizing vasculitis). Frequently, retinal tissue surrounding the most severely affected vessels is edematous and contains infiltrates of small to large numbers of degenerate neutrophils admixed with hemorrhage, necrotic cellular debris, and fibrin (ischemic necrosis). The subretinal space and vitreous contain small numbers of neutrophils admixed with hemorrhage and large amounts of loose fibrin. Free floating within the anterior chamber and disrupting and infiltrating the ciliary body at the iridocorneal angle are small to moderate amounts of fibrin admixed with small numbers of viable and degenerate neutrophils.

Contributor's Morphologic Diagnoses:
1. Right eye: Severe, multifocal to coalescing, acute necrohemorrhagic chorioretinitis and anterior uveitis with necrotizing fibrinoid vasculitis, fibrin thrombi, and intralesional gram-negative coccobacilli.
2. Right eye: Moderate, multifocal to locally extensive, acute to subacute hypopyon.

Contributor's Comment:
Histophilus somni (formerly *Haemophilus somnus*) is an economically significant disease of cattle and, to a lesser extent, sheep. Part of the Bovine Respiratory Disease Complex, it is associated with acute, fulminant shipping fever pneumonia primarily characterized by a fibrinous and suppurative bronchopneumonia with leukocyte necrosis, bronchiolar epithelial attenuation, and intravascular thrombi. While more commonly associated with pneumonia caused by *Mannheimia haemolytica*, oat cells are a commonly appreciated histologic feature.[4,9]

H. somni bacteremia, often a sequela to pneumonia, is reported to cause numerous and often severe changes in several tissues across multiple organ systems – termed the *H. somni* disease complex.[5,8,12,13] The most well-known manifestation of this syndrome is thrombotic meningoencephalitis (TME) characterized grossly by multiple foci of hemorrhage and necrosis throughout the brain and spinal cord, and histologically by necrotizing vasculitis with intravascular

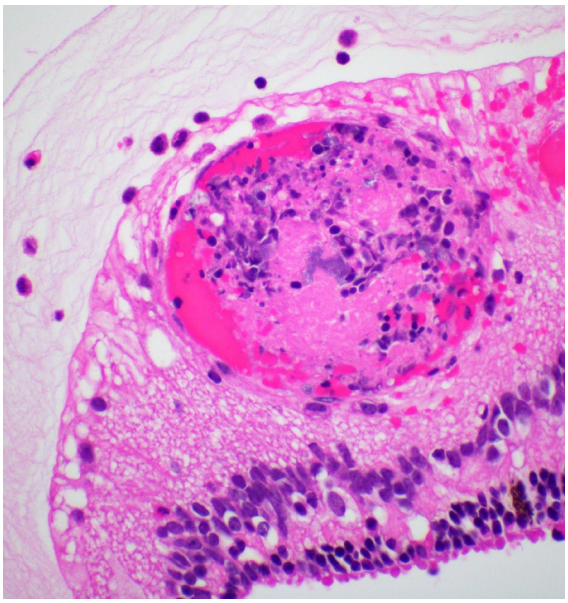

Figure 3-3. Retina, calf. Occasionally, colonies of coccobacilli are present within the thrombi (HE, 600X). (*Photo courtesy of:* Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK 74078 USA. https://vetmed.okstate.edu/veterinary-pathobiology/index.html)

thrombosis and ischemic necrosis of dependent tissues.[2,10] Other common disease entities secondary to bacteremia include otitis externa, laryngitis/tracheitis, myocarditis, metritis causing abortion or infertility, polyarthritis, mastitis, epididymitis, ampullitis, orchitis, and conjunctivitis.[2] The hallmark histologic lesion is vasculitis with secondary thrombosis, and colonies of gram-negative coccobacilli are commonly observed within vascular thrombi, affected blood vessels, and infarcted tissues most commonly affecting the central nervous system and retina, as observed in this case.[2]

Considered part of the normal flora of the male and female genital tracts and the nasal cavity, several mechanisms of transmission have been proposed.[5,8,12,13] Calves are suspected to become infected within the first few months of life via aerosolized urine and genital discharges. As with other pathogens of the bovine respiratory disease complex, infection is usually preceded by stressful events such as weaning, routine cattle processing, or shipping.[3,4,8,9,13] Synergism with concurrent viral infections, particularly Bovine Respiratory Syncytial Virus, in further facilitating lung damage, pneumonia, and bacteremia has been documented.[1,3] In cases of pneumonia, several virulence factors facilitating disease have been proposed including immunoglobulin Fc binding proteins (IgBPs) to resist serum and complement mediated killing; transferrin-binding proteins used in iron acquisition; biofilm formation; free oxygen-radical inhibition; and various other mechanisms of intracellular survival and immune evasion.[2,4,5,8,9,12,13] While evasion of bovine neutrophils and macrophages has been inconsistently reported as a potential virulence factor, the innate immune system (primarily via extracellular traps produced by neutrophils, macrophages, mast cells, and eosinophils) has been observed to successfully restrict the growth of *H. somni* in several anatomic locations, including the respiratory system.[8]

In cases of vasculocentric pneumonia or vasculitis secondary to bacteremia, surface lipooligosaccharide (LOS) has been proposed as a significant virulence factor. LOS has been demonstrated *in vitro* to induce endothelial cell apoptosis via activation of caspase 3, triggering the production of reactive oxygen species and nitrogen intermediates.[2,5,12,13] Another pathway suggests LOS triggers platelet activation leading to endothelial apoptosis via caspases 8 and 9, endothelial cell cytokine production and adhesion molecule expansion, and promotion of endothelial cell production of reactive oxygen species which further enhances endothelial apoptosis.[2] LOS has been demonstrated to undergo

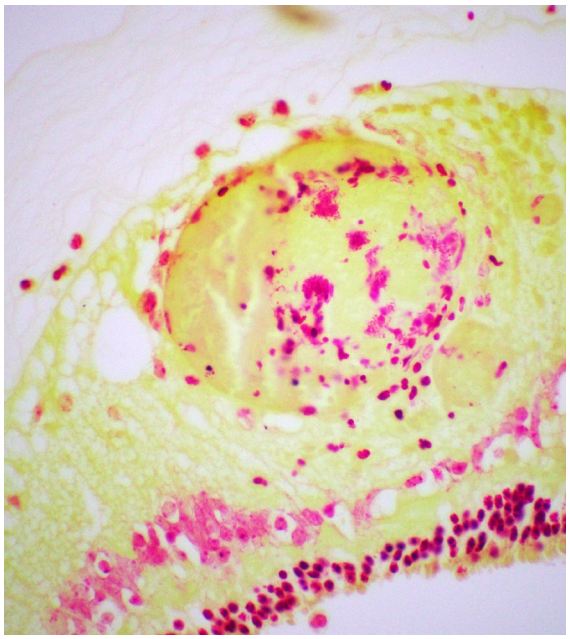

Figure 3-4. Retina, calf. A Gram stain demonstrates gram-negative coccobacilli. (Gram, 500X) (*Photo courtesy of:* Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK 74078 USA. https://vetmed.okstate.edu/veterinary-pathobiology/index.html)

phase and antigenic variation and sialylation contributing to immune resistance.[5,13] It has been proposed that predisposed sites of infarctive lesions and vasculitis include those in which there are dramatic changes in blood vessel diameter or flow patterns that increase the likelihood of endothelial or platelet activation.[10]

In the present case, necrotizing and fibrinoid vasculitis with thrombosis and tissue infarction was observed within the cerebrum, cerebellum, brainstem, right eye, and left ventricle and papillary muscle of the heart. In addition, there was a severe fibrinosuppurative bronchopneumonia, right ventricular endocarditis, fibrinosuppurative otitis media, and multifocal fibrinosuppurative polyarthritis. Samples of lung and brain were positive for *H. somni* on PCR. *Mycoplasma bovis* was an important differential diagnosis in this case, and PCR testing was positive for *M. bovis* in the lung but negative in the synovial fluid of the joints.

Contributing Institution:
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JPC Diagnosis:
Eye, retina and uvea: Vasculitis, necrotizing, multifocal, with fibrin thrombi, coccobacilli, and mild neutrophilic endophthalmitis.

JPC Comment:
Ocular histophilosis is a less common presentation of thrombotic meningoencephalitis (TME) in cattle; when present, however, the lesions are identical to the common, well-described lesions found in the brain of affected animals.[15] Ocular histophilosis commonly presents with suppurative thrombotic lesions in the choroid and the retina, often with intravascular bacterial colonies visible on H&E.[6,15] Ocular histophilosis can also present with retinitis as the sole ocular lesion, a histologic presentation shared with toxoplasmosis and infections with neurotropic viruses.[15] The presence of ocular lesions is useful as an aid in clinical diagnosis of TME and is estimated to occur in 30-50% of animals with the septicemic form of the disease.[15]

The contributor provides an excellent overview of the typical disease syndromes of histophilosis and of the virulence factors which cause its characteristic lesions. In addition to LOS, the key virulence factor discussed above, *H. somni* appears able to form biofilms on the endothelial surfaces of the microvasculature, particularly in the heart and

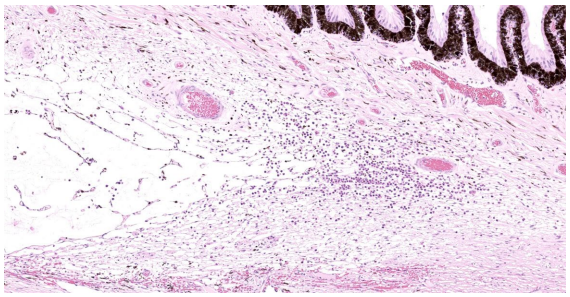

Figure 3-5. Drainage angle, calf. There is an accumulation of neutrophils and small amounts of fibrin in the drainage angle, likely the result of accumulation from low-grade anterior uveitis. (HE, 400X)

the brain.[11] Biofilms are collections of bacteria enclosed in a matrix composed of extracellular polysaccharides, proteins, and nuclei acids. The matrix allows cells to adhere to each other and/or other surfaces and allows for metabolic cooperation among the colony.[11] The matrix also provides protection from antibacterial agents, with bacteria within biofilms being less susceptible to antimicrobial therapy.[11] It is unknown whether biofilm formation occurs within ocular histophilosis, but biofilm formation and the subsequent concentration of endotheliotoxic virulence factors appears to be a significant contributor to pathogenesis in cardiac histophilosis and TME.[11]

Conference discussion initially revolved around differential diagnoses, with the moderator gently prodding residents to recall *Chlamydia pecorum*, which can cause many of the same clinical signs and histologic lesions as *H. somni*. *Chlamydia pecorum* can be cound in aborted fetuses, calves, and cows, and can cause meningoencephalitis, thrombosis, vasculitis, pericarditis, and polyserositis, with intracytoplasmic bacteria in endothelial and mononuclear cells visible on histology.[14]

Dr. Neto discussed the various manifestations of histophilosis and noted that a negative culture does not necessarily rule out histophilosis. A recent study found that culture of *Histophilus somni* from infection-confirmed tissue is often unsuccessful, making PCR the preferred diagnostic modality.[7] Finally, Dr. Neto discussed the very nature of the thrombus itself, providing examples of fibrin-rich thombi, with the expected PTAH stain positivity, and platelet-rich thrombi, which did not stain with PTAH. Immunohistochemical staining with CD61, which forms part of the platelet fibrinogen receptor glycoprotein IIb/IIIa, can be used to identify these platelet rich, PTAH-negative thrombi in formalin-fixed tissues.

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CASE IV:

Signalment:
11-year-old spayed female Maine Coon cat (*Felis catus)*

History:
This animal had access to the external environment and exhibited a recognized propensity for predation. No other cats shared the same household. The cat presented with multiple indolent nodular skin lesions ranging from 5 to 20 mm in diameter around both eyes, on the right ear-base region, and on the ventral aspect of the tongue, as well as an ulcerated skin nodule on the tail. The cat was referred to a veterinary ophthalmologist. A cutaneous mycobacteriosis was diagnosed through cytological and histological examination. Surgical excision accompanied by a 4-week course of antimicrobial therapy (rifampicin combined with clarithromycin) was implemented. A culture followed by the molecular characterization of the isolate identified *Mycobacterium lepraemurium* in the resected periocular nodule.

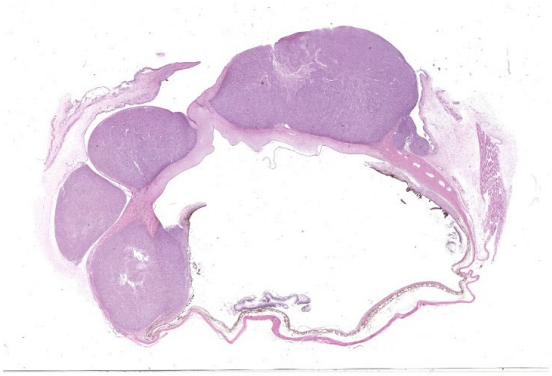

Figure 4-1. Eye, cat. There are multiple inflammatory nodules extending from the cornea, limbal conjunctiva, and one nodule (lower left) infiltrates the sclera. (HE, 5X).

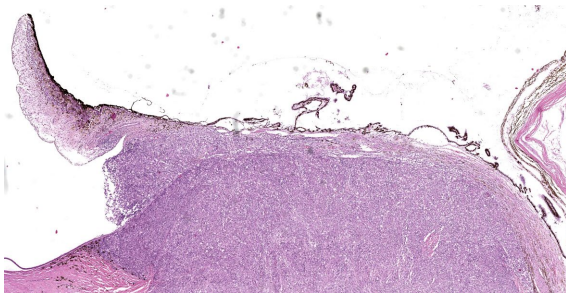

Figure 4-2. Eye, cat. The inflammatory nodule infiltrating the sclera extends into the drainage angle, uvea, and choroid. (HE, 60X)

The animal remained free from recurrences for at least one year following the surgical intervention; however, the cat subsequently experienced a relapse and was euthanized. The case was referred to the Institute of Veterinary Pathology of Zurich (IVPZ) for necropsy.

Gross Pathology:
The skin and subcutis, particularly on the head, dorsal region, distal limb area, and the periocular region, conjunctiva, and cornea, presented numerous randomly distributed, well-circumscribed, alopecic, beige, soft, elevated nodules with sizes varying between 0.2 and 1.5 cm in diameter. The nodules in the rostral facial region were infiltrating and destroying the osseous structures (os nasale, os incisivum, nasal conchae).

Microscopic Description:
Right eye: Within the cornea and sclera, infiltrating and expanding the ciliary body and the iris and obliterating the irido-corneal angle with focal disintegration of Descemet's membrane are multiple, well-circumscribed, coalescing granulomas ranging up to 1.2 cm in diameter. The granulomas contain numerous epithelioid macrophages, occasional multinucleate giant cells (foreign body type), scattered small lymphocytes, plasma cells, fibroblasts, fibrous connective tissue, and neutrophils. Occasionally, nodules contain a central area of necrosis characterized by abundant eosinophilic cellular and karyorrhectic debris. The cytoplasm of macrophages contains numerous negative staining rod-shaped structures. The corneal epithelium, which lines the distended corneal stroma affected by granulomas, exhibits multifocal areas of ulcerations characterized by loss of epithelial lining cells. Numerous smaller granulomas are present interspersed among the connective tissue of the eyelid and conjunctiva.
A Ziehl-Neelsen acid fast stain revealed myriad intrahistiocytic and free, 0.5 x 2 µm, acid-fast filamentous bacilli.

Contributor's Morphologic Diagnosis:
Globe: Severe, multifocal to coalescing, chronic granulomatous keratoconjunctivitis and anterior uveitis with numerous intrahistiocytic and extracellular acid-fast bacilli.

Contributor's Comment:
Mycobacteria in cats can have different clinical presentations, ranging from localized cutaneous lesions to generalized, systemic infections. Mycobacteria are categorized into three main groups.

The ***Mycobacterium tuberculosis*** **complex (MTBC)** contains 9 closely-related host adapted species with varying ability to cross species barriers. Among cats, the most prevalent species from this group (in the UK) are the rodent-adapted *Mycobacterium microti* and the cattle-adapted *Mycobacterium bovis*.[6] The lesions caused by MTBC are usually present in skin and lymph nodes as an effect of bite and fighting wounds, but may also be present in the lungs, gastrointestinal tract, and rarely, joints.[8]

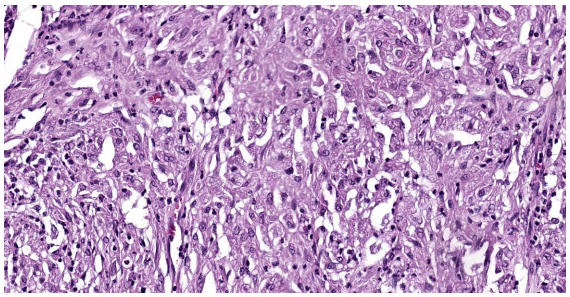

Figure 4-3. Eye, cat. The inflammatory nodules are composed of innumerable epithelioid macrophages with fewer lymphocytes, plasma cells, and neutrophils. (HE, 311X)

Non-tuberculosis mycobacteria (NTM) include mainly opportunistic organisms that may be found in the environment, including water, soil, aerosols, and vegetation. In cats, the species of main concern are *Mycobacterium malmoense*, *Mycobacterium branderi/ shimoidei,* and *Mycobacterium avium.* The infections caused by pathogens from this group are usually systemic and have a high shedding potential given the fact that extracellular bacilli are found in respiratory and intestinal epithelium as well as in glomerular tufts.[7]

The ***Mycobacterium leprae*** **complex (MLC)** causes "feline leprosy syndrome" (FLS) and includes *M. lepraemurium, M. visibile*, and *Mycobacterium* sp. strain Tarwin. The standard microbiological methods are not effective for the culture of these species. Clinical presentation of the infection is usually limited to skin, and the dermal lesions are nodular, often ulcerated, and present predominantly on the head and the forelimbs. The generalized nodular skin disease consists of numerous disseminated skin nodules and is a rarely observed pattern of infection. Despite the predisposition of *M. lepraemurium* to cause damage in the head area, it does not tend to involve the eyes. In contrast, *Mycobacterium* sp. strain Tarwin can cause feline leprosy with a particular propensity to produce lesions on the head, particularly involving the eyes and periocular skin.[5]

Histologically, the lesions seen in cats affected by feline leprosy syndrome can be divided into two categories. Lepromatous leprosy is thought to be the malignant form and is accompanied by numerous, typically intracellular acid-fast bacilli (multibacillary), whereas the tuberculoid leprosy is frequently recognized as the benign form of the disease and is defined by the presence of relatively small numbers of intrahistiocytic acid-fast bacilli (paucibacillary).[2]

The most likely method of transmission for *M. lepraemurium*, which causes leprosy in cats and rodents, is through the bites of infected prey during hunting. It has not yet been established whether there is a niche in the environment where the pathogen can survive without its host; however, the clinical and histological findings in wild rodents, where the pathogen is well adapted, and cats serving as incidental hosts seem to support this hypothesis.[1]

Contributing Institution:
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Vetsuisse-Fakultät, Universität Zürich
https://www.vetpathology.uzh.ch

JPC Diagnosis:
Eye: Keratoconjunctivitis, scleritis, and uveitis, pyogranulomatous, multifocal to coalescing, severe.

JPC Comment:
As the contributor notes, *Mycobacterium lepraemurium* does not typically involve the ocular tissues; in fact, this is the only case of ocular mycobacteriosis due to *M. lepraemurium* reported in Europe to date.[1,3] In a recent

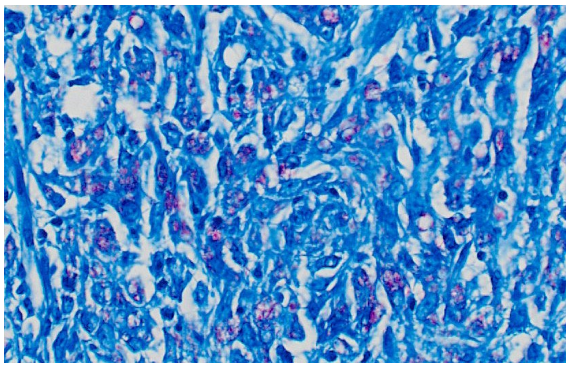

Figure 4-4. Eye, cat. An acid-fast stain demonstrates acid-fast bacilli in numerous macrophages within the inflammatory infiltrate. The inflammatory nodules are composed of innumerable epithelioid macrophages with fewer lymphocytes, plasma cells, and neutrophils. (Fite-Faraco, 400X).

study of ocular mycobacteriosis in cats, tuberculosis (infection with *Mycobacterium bovis, Mycobacterium microti*, or a nonspeciated *Mycobacterium tuberculosis* complex pathogen) was diagnosed in 91% of cats, while *Mycobacterium lepraemurium* was identified in only 9% of cats, providing further evidence for the relative rarity of this pathogen in ocular tissues.[3] The study found that the choroid, retina, ciliary body, and sclera were the tissues most affected in ocular disease caused by mycobacterial organisms other than *M. lepraemurium*, with inflammation being most florid within the choroid.[3] By contrast, the two ocular cases of *M. lepraemurium* infection contained lesions restricted to the cornea, sclera, and conjunctiva.[3] Infection restricted to the external tunic of the eye is typical of this infection and may be due to the inability of *M. lepraemurium* to replicate well at higher body temperatures.[3]

The division of feline leprosy syndrome into lepromatous or tuberculoid forms is based on the differential elaboration of canonical cytokines by CD4+ T lymphocytes. In the tuberculoid form, Th1 lymphocytes produce large amounts of IL-2 and interferon gamma, resulting in a cell-mediated immune response rich in activated macrophages and T-cells which, in a virtuous immune feedback loop, stimulate mutual proliferation and activation and the subsequent containment of infection.[4] Lesions of this type are paucibacillary due to the low numbers of surviving infectious organisms.

By contrast, lepromatous disease produces multibacillary lesions that are driven by a Th2 lymphocyte response. In these cases, the Th2 lymphocytes produce a cytokine milieu rich in IL-4, IL-10, and IL-13, all of which suppress macrophages and stimulate B lymphocytes, and IL-5, which recruits eosinophils. The resulting humoral response stifles cell-mediated immunity and allows infections to progress, resulting in more diffuse, multibacillary infections such as that present in this case.[4] In vivo, these responses do not exist as discrete entities, but rather occur on a continuum with the relative contribution of Th1 and Th2 responses driving disease progression and clinical outcomes. In fact, the recent study discussed above found that high bacterial loads were found with infections with *M. bovis, M. microti* and *M. lepraemurium*, indicating that the presence of multibacillary lesions is not necessarily restricted to infections with non-tuberculosis mycobacteria and *Mycobacterium leprae* complex pathogens.[4]

Conference participants discussed the origin of these stiking lesions and debated whether the infection was of hematogenous or external, “outside-in” origin. Given the gross appearance and the nodules arising from the conjunctiva and cornea, participants decided that an external origin was most likely. Par-

ticipants also discussed whether the discontinuity in Descemet's membrane was real or artifactual, before deciding that the lesion is real due to the highly reactive underlying stroma. Elsewhere in the cornea, some loss of stromal clefting was noted, but Dr. Neto cautioned resident against overinterpreting corneal edema in a necropsied animal as some post-mortem corneal aqueous absorption is common.

Dr. Neto also discussed the choice of carbolfuchsin stains for acid-fast organisms. In general, she finds Ziehl-Neelsen to be less effective for non-tuberculous *Mycobacterium* as the solvent used may rapidly remove dye from the organisms' thinner walls; however, Fite-Faraco has can cause indiscriminate staining of necrotic material, making Ziehl-Neelsen the stain of choice in the face of abundant necrosis.

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