The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator:
Shannon Lacy, DVM, MPH

WEDNESDAY SLIDE CONFERENCE 2009-2010

Conference 6

21 October 2009

Conference Moderator:

Dr. Dale G. Dunn, DVM, Diplomate ACVP

CASE I: 08N2533 (AFIP 3134342).

Signalment: 7-month-old spayed, female Maltese mix dog (*Canis familiaris*).

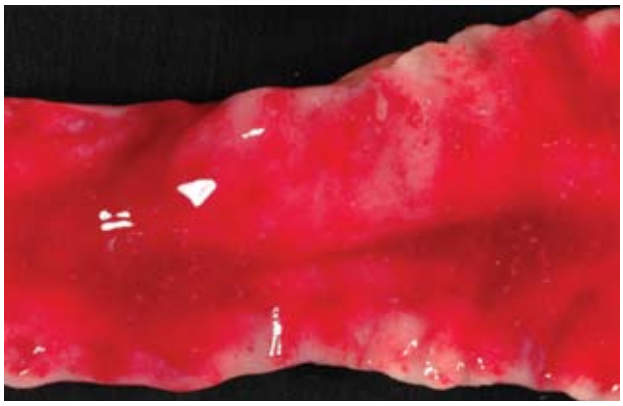
History: This dog presented with a 5-day history of lethargy and 2-day history of anorexia and vomiting. She was up to date on vaccines and had no prior medical problems. Physical exam: The dog was obtunded and laterally recumbent. Mucous membranes were pale and tacky with prolonged capillary refill time. She had moderate tachycardia with synchronous, weak femoral pulses. She was hypothermic with a rectal temperature of 96.8 degrees Fahrenheit. Dehydration was assessed as 8-10%. Following rehydration, the dog developed watery melena and vomited frank blood.

Gross Pathology: The duodenum was thickened (3 mm) and mottled dark red and tan on the serosal surface. Gastric and intestinal contents were dark red and mucoid. Scattered throughout the gastrointestinal tract, but noted most prominently in the gastric cardia and fundus, duodenum, and colon, were numerous (>100) 1 mm diameter, raised, pale, tan nodules with pinpoint central depressions (**fig. 1-1**). The splenic surface was irregular with shallow 2-3 mm pits over the entire surface. There was petechiation on the capsular surface of an enlarged mesenteric lymph node (approximately twice normal size). On cut surface, the medulla and part of the cortex was dark red (hemorrhage); the remaining cortex was pale pink (**fig. 1-2**).

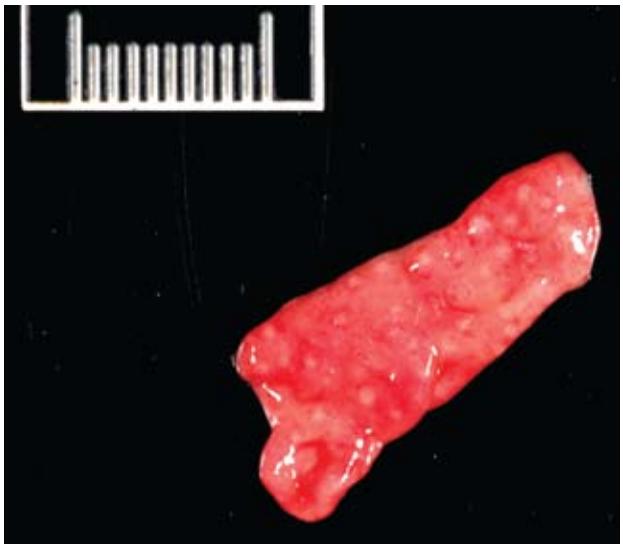
Laboratory Results: **Bloodwork** revealed elevated packed cell volume, increased blood urea nitrogen, and mild hyponatremia, hypokalemia, and hypochloremia. **Abdominal radiographs** showed increased soft tissue density in the ileocecocolic region, but were otherwise unremarkable. **Parvovirus fecal antigen test:** negative. **Fecal flotation:** *Nanophyetus salmincola* eggs isolated.

Histopathologic Description: **Mesenteric lymph node:** In this lymph node, follicles are absent and subcapsular and medullary sinuses are filled with numerous macrophages that have abundant eosinophilic cytoplasm and frequently contain numerous basophilic, less than 1 um diameter, coccoid to coccobacillary organisms surrounded by a thin clear space or occasionally forming clusters within vacuoles (**figs. 1-3 and 1-4**). Smaller numbers of macrophages are scattered through the cortex, where there are multifocal to coalescing areas of necrosis. Increased numbers of plasma cells are present throughout the cortex and medullary cords.

*Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists, and the C. L. Davis Foundation.



1-1. Duodenum, dog. The duodenum is thickened and mottled dark red and tan with scattered 1 mm diameter raised pale nodules with pinpoint central depressions. Photographs courtesy of University of California, Davis, Veterinary Medical Teaching Hospital, One Shields Ave, Davis, CA 95616, *rmgriffey@ucdavis.edu.*



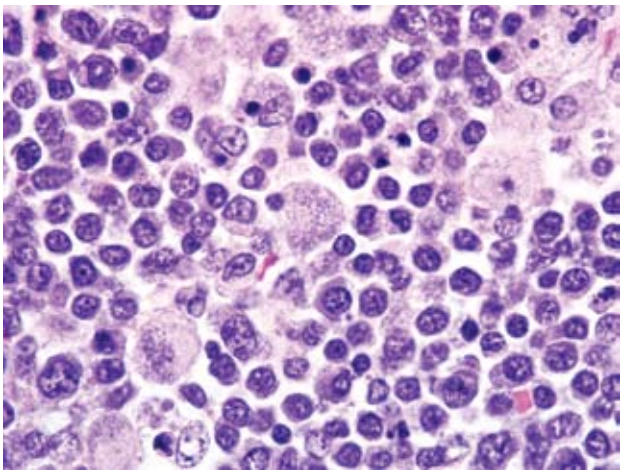
1-2. Lymph node, dog. On cut surface, the medulla and part of the cortex are hemorrhagic. Photographs courtesy of University of California, Davis, Veterinary Medical Teaching Hospital, One Shields Ave, Davis, CA 95616, rmgriffey@ucdavis.edu.

Duodenum: In this section of duodenum, there is generalized villous blunting with multifocal, epithelial necrosis and accumulation of sloughed cells within crypts (crypt abscesses). Embedded within the mucosa as well as within the intestinal lumen are multiple profiles of acoelomic parasites with a spiny cuticle, suckers, and operculated eggs (**fig. 1-5**). Some sections of individual parasites have both spermatids and ova (hermaphroditic). Moderate numbers of lymphocytes and plasma cells with fewer macrophages infiltrate the lamina propria and form a nearly circumferential band between the muscularis mucosa and the base of villi. Macrophages containing clusters of the same intracellular organisms observed in the lymph node (described above) are occasionally noted within the muscularis mucosa and submucosa. Mucosal epithelium is alternately attenuated to regenerative with some cells exhibiting large, vesicular nuclei. There are scattered regions of submucosal hemorrhage.

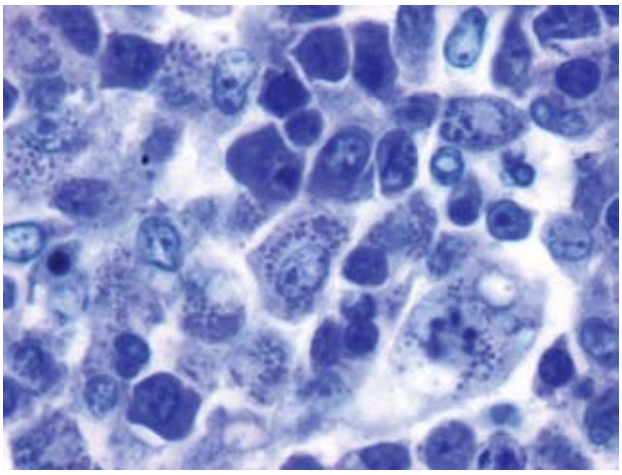
Special stains: The organisms seen within macrophages on H&E stained red (gram-negative) with Brown and Brenn and purple-blue with Giemsa stains. Machiavello's stain was inconclusive.

Contributor's Morphologic Diagnosis: 1. Mesenteric lymph node: Severe, multifocal to coalescing granulo–matous and necrotizing lymphadenitis with intracellular rickettsiae (*Neorickettsia helmintheoca*, presumptive).
2. Duodenum: Moderate, multifocal, chronic, granulo–matous enteritis with intracellular rickettsiae.
3. Duodenum: Trematodiasis (*Nanophyetus salmincola*).
4. Duodenum: Moderate, diffuse, chronic, lympho–plasmacytic enteritis.

Contributor's Comment: This case presents some of the classical clinical signs and gross and histopathologic lesions of salmon poisoning disease caused by *Neorickettsia helminthoeca*, a 0.3 um coccoid to coccobacillary, gram-negative, obligate intracellular bacteria, which occasionally forms rods up to 2 um long.[3] *N. helminthoeca* is transmitted via the helminth *Nanophyetus salmincola*, a trematode whose life cycle requires two intermediate hosts and one definitive host. Development from miracidia to cercariae occurs within the first intermediate host, a snail (*Oxytrema silicula)*. Cercariae are then released into the water where they infect a secondary intermediate host via skin penetration. Salmonid fish are the typical secondary host, but infection of some non-salmonid fish and the Pacific giant salamander does occur.[3] Cercariae develop to metacercariae and localize mainly in the kidneys and skeletal muscle, although they may be found in any organ.[1] Transmission to the definitive host follows ingestion of an infected fish. Adult trematodes then develop within the intestine where they attach to the mucosa and ova are released to the environment in feces, generally in 5-7 days post-infection.[3] *N. helminthoeca* is transmitted transovarially within the trematode and is present in all life cycle stages.[7] It is inoculated by unknown means into the intestines of definitive hosts, where replication



1-3. Lymph node, dog. Multifocally within medullary sinuses are high numbers of histiocytes with abundant eosinophilic cytoplasm and frequently containing numerous basophilic, <1 um rickettsiae surrounded by a thin clear vacuole. (HE 1000X)

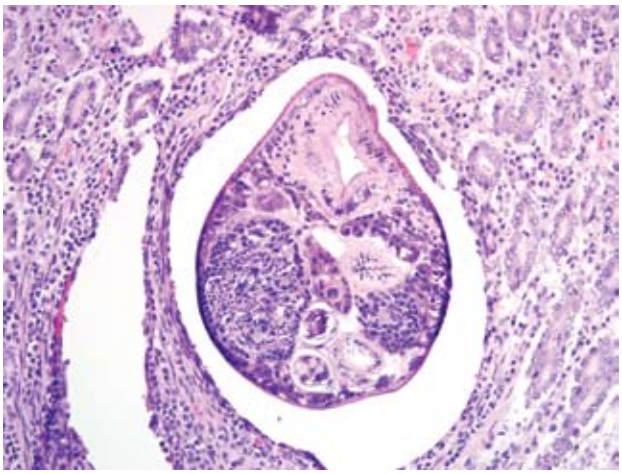


1-4. Lymph node, dog. Intrahistiocytic rickettsiae stain purple-blue with Giemsa stain. Photomicrograph courtesy of University of California, Davis, Veterinary Medical Teaching Hospital, One Shields Ave, Davis, CA 95616, rmgriffey@ucdavis.edu.

initially occurs in either intestinal epithelium or lymphoid follicles.[3] Definitive hosts of the trematode may be any fish-eating bird or mammal. There are a number of known natural avian and mammalian definitive hosts; however, primarily canid species, including domestic dogs, coyotes, and foxes, are susceptible to *N. helminthoeca* infection.[3] In addition, several species of captive bears have been reported that developed clinical signs of salmon poisoning disease following ingestion of raw or improperly stored fish from the endemic area and were passing *N. salmincola* eggs.[2,7]

Salmon poisoning disease generally has an incubation period of 5-7 days and common clinical signs are fever, anorexia, vomiting, diarrhea, and lymphadenopathy.[3] Bloody diarrhea and hypothermia may develop in the late stages of the disease, as was the case in this dog. The presence of the fluke is generally of little clinical significance.[3] If left untreated, the disease has a high mortality rate; the specific cause of death is unknown. *Neorickettsia* are able to evade the immune system by inhibition of lysosomal fusion with its parasitophorous vacuole, a process which has been shown to be reversed by oxytetracycline treatment.[9] Accordingly, appropriate treatment of the disease warrants an excellent prognosis. In one study, 91% of naturally infected dogs treated with tetracycline made a full recovery.[8] Recovered animals generally develop immunity.[3]

Antemortem diagnosis is based on history and typical clinical signs along with presence of *N. salmincola* ova on



1-5. Small intestine, dog. Embedded deep within an intestinal crypt is a cross section of an adult trematode. (HE 200X)

fecal flotation.[8] Further confirmation is provided by lymph node aspirates showing macrophages with rickettsia-like cytoplasmic inclusions. The organisms stain purple with Giemsa, red with Macchiavello's, black or dark brown with Levaditi's method, and pale blue with hematoxylin and eosin. Peripheral lymphadenopathy was not present in this case. On necropsy, gross lesions typically involve the lymphoid tissues, spleen, and gastrointestinal tract. These include lymphoid tissue enlargement, lymph node petechiation, splenomegaly, and petechiation and ulceration in the gastrointestinal tract.[3] Similar gross findings were noted in the present case, with the addition of gross thickening of the duodenum and pinpoint raised

mucosal nodules in the stomach, duodenum, and colon, which corresponded histologically to lymphoid follicles and inflammatory nodules in which there were macrophages with intracellular rickettsiae. The presence of the rickettsiae in macrophages within the stomach wall has not been reported. Given that *N. helminthoeca* is presumed to be inoculated into the intestinal mucosa, its presence in the stomach suggests either inoculation in gastric mucosa as well, or a particular affinity for this tissue during systemic distribution. Diagnosis in this case was facilitated by the finding of classic microscopic lesions. In the lymphoid tissues, these are depletion of mature lymphocytes, foci of necrosis, and hyperplasia of mononuclear phagocytes with intracytoplasmic rickettsia-like organisms. Trematodes are found embedded in the intestinal mucosa, primarily in the duodenum, as was the case here. Nonsuppurative meningitis or meningoencephalitis has been reported experimentally, but neither was observed in this case.[4]

The endemic area of *O. silicula*, the first intermediate host of the trematode, ranges from southern Vancouver Island as far south as Lake Tahoe along the western slopes of the Cascade and Sierra mountains.[7] The natural habitat of the snail used to define the affected area, but stocking of sport fisheries and other public waters with infected fish from both state and private hatcheries, as well as natural movement of salmonid fish to new areas, has expanded the range of infected fish, thereby expanding the range of potential cases of salmon poisoning disease.[7] In the present case, further questioning of the owner revealed that the family had eaten trout from a region in the Sierra foothills southwest of Lake Tahoe, which could represent the source of exposure for this dog. Interestingly, salmon poisoning or a similar disease could be emerging in an entirely separate region. There are reports from southern Brazil of a disease in dogs with gross and histopathologic lesions similar to salmon poisoning disease.[6] In these cases, intracytoplasmic organisms were observed, and trematodes were present in the large intestine of one dog that were considered consistent with *Ascocotyle arnaldoi*, a trematode that also requires both a snail and fish intermediate host. Preliminary PCR studies have isolated gene fragments with close homology to *N. helmintheoca* from tissues of two such affected dogs.[5]

AFIP Diagnosis: 1. Duodenum: Enteritis, lympho–histiocytic and plasmacytic, diffuse, moderate, with villar blunting and fusion, crypt hyperplasia and abscesses, fibrinous vasculitis, hemorrhage, and numerous intra–histiocytic rickettsiae.
2. Duodenum: Intramucosal adult trematode.
3. Lymph node, mesenteric (per contributor): Lymphadenitis, necrotizing and histiocytic, multifocal to coalescing, moderate, with plasmacytosis, and numerous intrahistiocytic rickettsiae.

Conference Comment: This case is an excellent example of a classic entity, and the contributor provides a thorough review of the disease pathogenesis, clinical signs, gross and microscopic lesions, and epidemiology. In most conference participants' slides there are multifocal areas of hemorrhage within the submucosa and serosal adventitia of the duodenum. Vessels in these areas are frequently characterized by fibrinoid necrosis and mild vasculitis, as indicated in the AFIP morphologic diagnosis. Participants also noted moderate amounts of erythrophagocytosis by sinus histiocytes in the submitted lymph node. This is a common incidental finding in canine mesenteric lymph nodes.

Contributor: Anatomic Pathology Service, Veterinary Medical Teaching Hospital and Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, One Shields Avenue, Davis, CA 95616
http://www.vmth.ucdavis.edu
http://www.vetmed.ucdavis.edu/PMI

References:

1. Baldwin NL, Millemann RE, Knapp SE: Salmon poisoning disease. III. Effect of experimental *Nanophyetus salmincola* infection the fish host. J Parasitol **53**(3):556-564, 1967
2. Gai JJ, Marks SL: Salmon poisoning disease in two Malayan sun bears. J Am Vet Med Assoc **232**(4):586-588, 2008
3. Gorham JR, Foreyt WJ: Salmon poisoning disease. *In:* Infectious Diseases of the Dog and Cat, ed. Greene CE, 3rd ed. pp. 198-203. WB Saunders, Philadelphia, PA, 2006
4. Hadlow WJ: Neuropathology of experimental salmon poisoning of dogs. Am J Vet Res **18**(69):898-908, 1957
5. Headley SA, Scorpio D, Barat N, Vidotto O, Dumler JS: *Neorickettsia helminthoeca* in dog, Brazil. Emerg Infect Dis **12**(8):1303-1305, 2006
6. Headley SA, Vidotto O, Scorpio D, Dumler JS, Mankowski J: Suspected cases of *Neorickettsia*-like organisms in Brazilian dogs. Ann NY Acad Sci **1026**:79-83, 2004
7. Hedrick RP, Amandi A, Manzer D: *Nanophyetus salmincola*: the trematode vector of *Neorickettsia helminthoeca*. Calif Vet **44**:18-22, 1990
8. Mack RE, Bercovitch MG, Ling GV, Lobingier RT, Melli AC: Salmon poisoning disease complex in dogs – a review of 45 cases. Calif Vet **44**:42-45,1990
9. Rikihisa Y: Mechanisms to create a safe haven by members of the family Anaplasmataceae. Ann NY Acad Sci **990**:548-55, 2003

CASE II: 089-43900 (AFIP 3134372).

Signalment: 6-year-old castrated, male domestic shorthair cat (*Felis catus*).

History: Anorexia, soft tissue abdominal mass, no response to medical treatment.

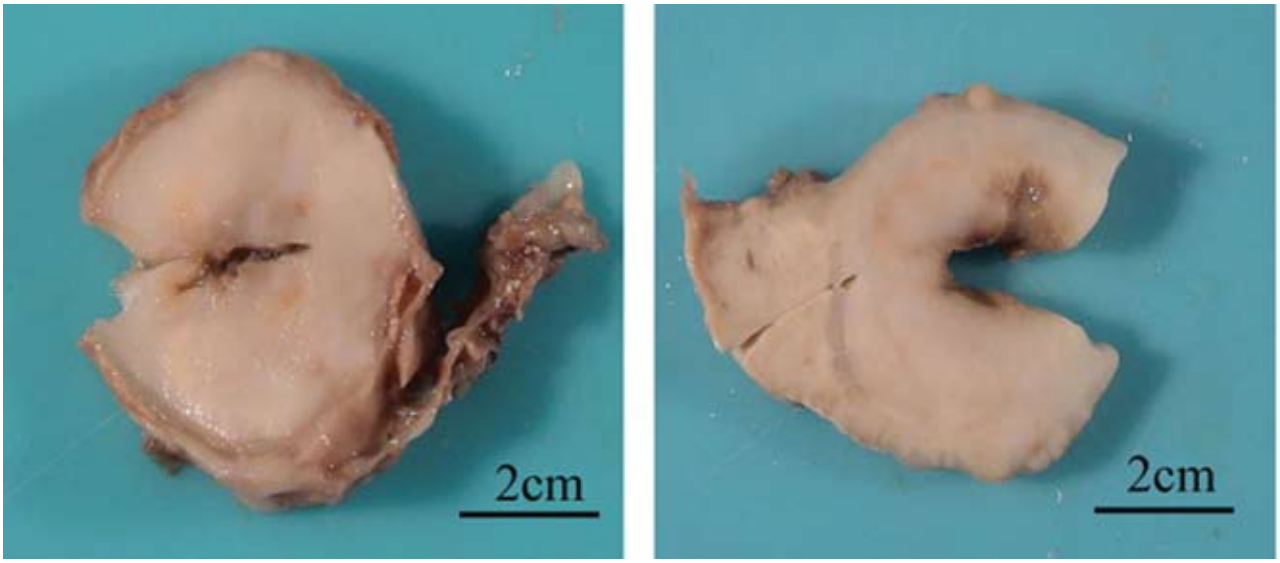
Gross Pathology: Jejunum: Marked transmural expansion by a firm, homogenous tan mass with mucosal ulceration (**fig. 2-1**).

Laboratory Results: Moderate leukocytosis and eosinophilia.

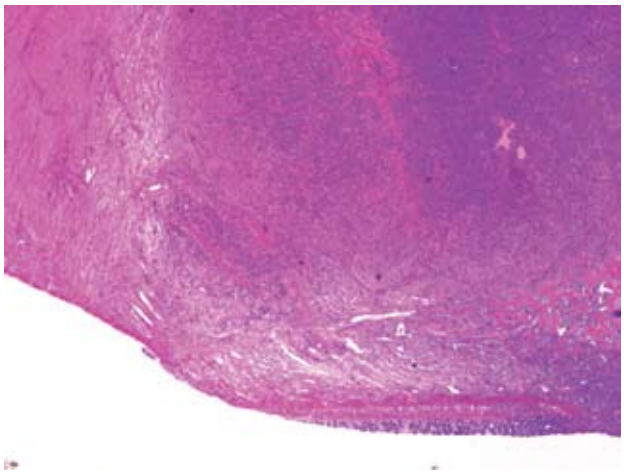
Histopathologic Description: Jejunum: Expanding and infiltrating the ulcerated mucosa, submucosa, tunica muscularis, and extending to the serosa is a poorly demarcated and unencapsulated mass. Neoplastic cells are arranged in a trabecular pattern separated, surrounded, and dissected by variably dense bands of stromal collagen (sclerosis). Neoplastic cell morphology varies from round to polygonal to spindled with variably distinct cell borders and a moderate amount of eosinophilic cytoplasm that very rarely contains basophilic granules. Nuclei range from round to oval to elongate with dispersed chromatin and one to two prominent nucleoli. Mitoses are infrequent, ranging from zero to one per 400X field. There is moderate to marked anisocytosis and anisokaryosis with occasional karyomegalic cells. Admixed throughout the neoplastic population are moderate to marked infiltrates of eosinophils.

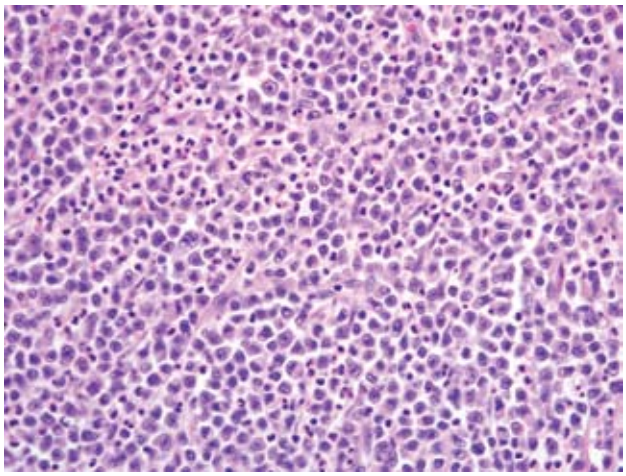
Contributor's Morphologic Diagnosis: Jejunum: Sclerosing mast cell tumor.

Contributor's Comment: This case was selected from a series of a unique and unreported sclerosing variant of feline intestinal mast cell tumor (MCT) recognized at the Colorado State University Veterinary Diagnostic Laboratory. Visceral forms of MCT are relatively common in the cat, with intestinal MCT being the third most common primary intestinal tumor following lymphoma and adenocarcinoma.[10] Furthermore, visceral forms (splenic and intestinal) of mast cell tumor are more often reported in the cat compared to the dog.[5] While these tumors typically display similar microscopic features regardless of anatomical location[7,8], a sub-group of intestinal MCT in the cat is presented here which is morphologically and biologically distinct from other mast cell tumors in this species, characterized by a significant stromal component. Masson's trichrome stain was pursued to confirm the stromal collagen composition of the sclerosing component, which is characterized by intense blue staining. Poorly discernible intracytoplasmic granules, which demonstrated metachromasia and enhanced visibility with giemsa and toluidine blue stains, suggest mast cell origin. Immunohistochemical staining for mast cell-specific tryptase further supported mast cell origin.[3]

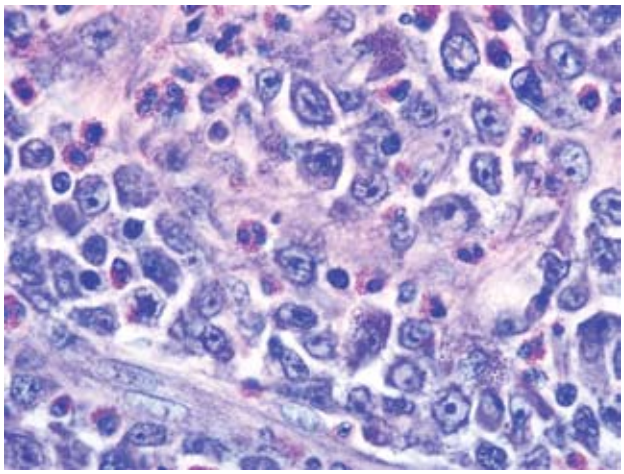


2-1. Jejunum, cat. There is marked transmural expansion by a firm, homogenous tan mass with mucosal ulceration. Photograph courtesy of Colorado State University, Department of Microbiology, Immunology, Pathology, 1619 Campus Delivery, Fort Collins, CO 80523, Karen.Fox@colostate.edu.





2-2, 2-3. Jejunum, cat. Infiltrating a focally extensive area of the submucosa and extending to the tunica muscularis is a moderately cellular, nodular proliferation of neoplastic round cells characterized by central nuclei, prominent nucleoli, moderate anisokaryosis, and a high mitotic rate. Fewer eosinophils are admixed with neoplastic cells. (HE 20X, HE 400X)

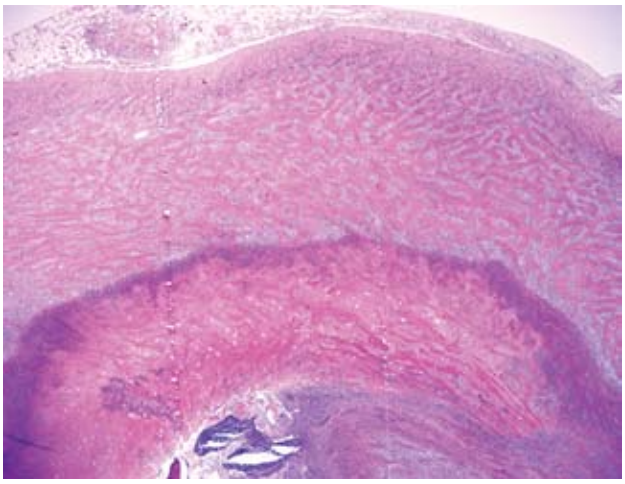


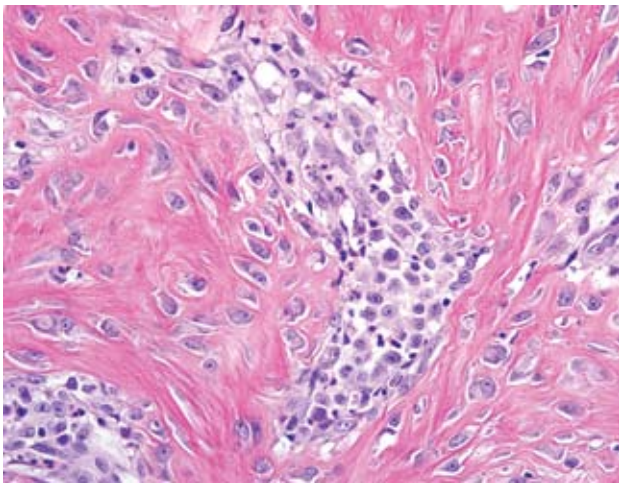
2-4. Jejunum, cat. Numerous non-neoplastic mast cells with metachromatic cytoplasmic granules are present within the nodular cellular proliferation. (Giemsa 1000X)

Howl and Petersen (1995) described a single case report of an intestinal mast cell tumor in a cat that presented with eosinophilic enteritis. In addition to the marked eosinophilic infiltrate, this case was characterized by a marked scirrhous response surrounding the neoplastic population similar to the case presented here.[6] This demonstrates the diagnostic challenge the eosinophilic infiltrate may introduce. Thus, when confronted with a significant eosinophilic intestinal population, close attention to all histological features is necessary in order to differentiate feline intestinal sclerosing mast cell tumors from other lesions such as eosinophilic granulomatous disease[6] and feline gastrointestinal eosinophilic sclerosing fibroplasia.[2] While the marked eosinophilic infiltrate in these tumors may pose a diagnostic challenge, the presence of eosinophils is not uncommon with mast cell disease. Mast cells promote eosinophilic inflammation by producing eosinophil-directed cytokines, such as IL-4 and IL-5. These cytokines subsequently induce chemokines that specifically attract eosinophils, such as eotaxin-1 and 2.[9] In addition, the mucosal ulceration may be due, in part, to local vasoconstriction in response to the release of vasoactive substances from the mast cells, such as histamine, and subsequent devitalization of the overlying mucosal epithelium as well as by direct infiltration and mucosal effacement by the neoplastic cells.

The sclerotic component was a unique feature in all cases evaluated. There is increasing evidence that mast cells play a central role in tissue remodeling in chronic inflammatory reactions. Among the numerous substances released by mast cell tumors are the fibrogenic cytokines fibroblast growth factor and transforming growth factor β1.[1,4] These cytokines promote activation, proliferation, and migration of fibroblasts with subsequent collagen production and contraction. In addition to mast cells, eosinophils have also been shown to play a role in fibrosis. Eosinophils are similarly involved in tissue remodeling and fibrosis in allergic reactions via the release of fibrogenic cytokines such as TGF-β and IL-1β.[2,4] The release of these fibrogenic cytokines by mast cells and/or eosinophils suggests a possible mechanism of mast cell-induced sclerosis in this tumor.

AFIP Diagnosis: 1. Jejunum: Malignant round cell neoplasm.

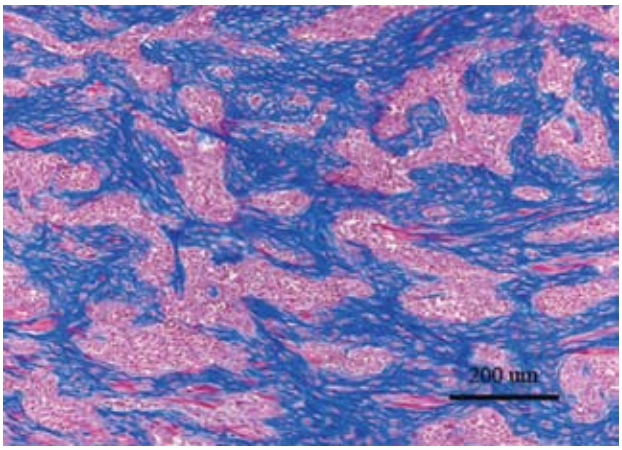




2-5, 2-6. Jejunum, cat. Multifocally within the submucosa and tunica muscularis are numerous evenly-spaced, anastomosing trabeculae of dense fibrous connective tissue interspersed with a moderate cellular infiltrate. Trabeculae of fibrous connective tissue are surrounded by reactive fibroblasts. The cellular infiltrate is composed of numerous non-neoplastic mast cells, macrophages, and fewer eosinophils. (HE 20X, HE 400X)

2. Jejunum: Enteritis, ulcerative, eosinophilic and mastocytic, sclerosing, transmural, severe, with mixed bacteria and anisotropic foreign material.

Conference Comment: This very intriguing case stimulated a vibrant discussion during the conference. While conference participants carefully considered the contributor's diagnosis of mast cell tumor, most participants favored the less specific diagnosis of malignant round cell neoplasm (**figs. 2-2 and 2-3**). Further, most participants considered the malignant neoplasm to be a separate, albeit possibly related, lesion from the eosinophilic and sclerosing fibroplasia. At the AFIP, additional histochemical and immunohistochemical stains did not further elucidate the histogenesis of the neoplasm. The Giemsa and toluidine blue histochemical stains demonstrated numerous metachromatic cytoplasmic granules within many mast cells throughout the areas of sclerosis in the section (**fig. 2-4**); the mast cells were interpreted as non-neoplastic, as they lack features of malignancy and are relatively evenly spaced within bands of collagen and fibroblasts (**figs. 2-5, 2-6, and 2-7**). Furthermore, metachromatic granules were not demonstrable in the population interpreted as neoplastic cells, i.e. the sheets of monomorphic round cells with atypia and increased mitotic rate. Immunohistochemical staining with CD117a (c-kit) was noncontributory. While poorly-differentiated mast cell tumor with lack of typical cytoplasmic granules remains a possibility, many participants considered lymphoma in the differential diagnosis and attributed the prominent eosinophilic infiltrate secondary to cytokines released by neoplastic lymphocytes. Immunohistochemical staining with CD3 and CD79a revealed moderate numbers of intralesional T- and B-lymphocytes, respectively; however, the overtly neoplastic cells were negative for these markers. Based on histomorphology, and histochemical and immunohistochemical findings, participants ultimately favored the histologic diagnoses indicated above. This case demonstrates the diagnostic difficulties sometimes encountered when attempting to determine the histogenesis of poorly differentiated tumors.



2-7. Jejunum, cat. Masson's trichrome is staining stromal collagen (sclerosis) blue and cytoplasm red. Photomicrograph courtesy of Colorado State University, Department of Microbiology, Immunology, Pathology, 1619 Campus Delivery, Fort Collins, CO 80523, Karen.Fox@

The eosinophilic and sclerotic lesion in this case is remarkable, and the contributor offers a sound hypothesis

for its pathogenesis. In a recent case series involving 25 cats, Craig and co-authors[2] proposed the term "feline gastrointestinal eosinophilic sclerosing fibroplasia" for a distinctive gastrointestinal lesion characterized by the mural presence of thick trabeculae of collagen separated by dense aggregates spindled cells. As in the present case, eosinophils and mast cells were numerous, and evenly scattered throughout the lesion. Immunohistochemical positivity for vimentin and smooth muscle actin was consistent with myofibroblastic differentiation and the authors speculated that bacteria were important in the pathogenesis of this condition. Additionally, the authors hypothesized that some cats may have a genetic predisposition to the development of this lesion, as has been proposed for other feline eosinophilic inflammatory conditions, in response to a variety of antigens. As a result, eosinophilic sclerosing fibroplasia may be thought of as a nonspecific reaction pattern to foreign antigen in genetically susceptible cats. In this case, the round cell neoplasm may have incited eosinophilic sclerosing fibroplasia by a number of mechanisms. Neoplastic cells, regardless of origin, may have directly recruited eosinophils and fibroblasts through cytokine production as hypothesized by the contributor. Alternatively, the neoplasm may have rendered the intestine vulnerable to antigen exposure by causing ulceration, partial obstruction, or altered peristalsis, allowing bacteria and digesta to penetrate the mucosa. This is supported by the presence of ulceration, numerous mixed bacteria, and embedded anisotropic foreign material (i.e. hair and food material) in many of the sections. We thank the contributor for this interesting submission.

Contributor: Colorado State University, College of Veterinary Medicine and Biomedical Sciences
Department of Microbiology, Immunology, Pathology, 1916 Campus Delivery, Fort Collins, CO 80523
http://www.cvmbs.colostate.edu/mip/

References:

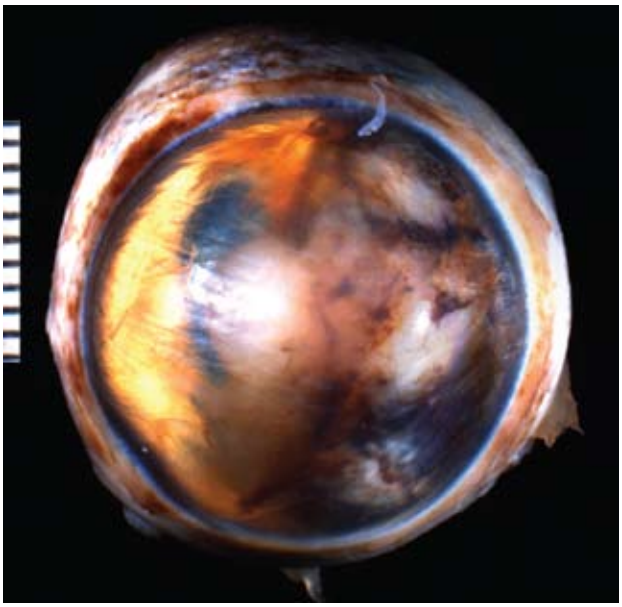
1. Batlle M, Pérez-Villa F, Lázaro A, Garcia-Pras E, Ramirez J, Ortiz J, Orús J, Roqué M, Heras M, Roig E: Correlation between mast cell density and myocardial fibrosis in congestive heart failure patients. Transplantation Proc **39:**2347-2349, 2007
2. Craig LE, Hardam EE, Hertzke DM, Flatland B, Rohrbach BW, Moore RR: Feline gastrointestinal eosinophilic sclerosing fibroplasia. Vet Pathol **46:**63-70, 2009
3. Fernandez NJ, West KH, Jackson ML, Kidney BA: Immunohistochemical and histochemical stains for differentiating canine cutaneous round cell tumors. Vet Pathol **42:**437-435, 2005
4. Gomes I, Mathur SK, Espenshade BM, Mori Y, Varga J, Ackerman SJ: Eosinophil-fibroblast interactions induce fibroblast IL-6 secretion and extracellular matrix gene expression: implications in fibrogenesis. J Allergy Clin Immuno **116:**796-804, 2005
5. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Feline mast cell tumors. *In:* Skin Diseases of the Dog and Cat, 2nd ed., pp. 858-861. Blackwell Publishing Co., 2005
6. Howl JH, Petersen MG: Intestinal mast cell tumor in a cat: presentation as eosinophilic enteritis. J Am Anim Hosp Assoc **31:**457-461, 1995
7. Johnson TO, Schulman FY, Lipscomb TP, Yantis TP: Histopathology and biological behavior of pleomorphic cutaneous mast cell tumors in fifteen cats. Vet Pathol **39:**452-457, 2002
8. Litster AL, Sorenmo KU: Characterisation of the signalment, clinical and survival characteristics of 41 cats with mast cell neoplasia. J Fel Med Surg **8:**177-183, 2006
9. Shakoory B, Fitzgerald SM, Lee SA, Chi DS, Krishnaswamy G: The role of human mast cell-derived cytokines in eosinophil biology. J Inter Cyto Res **24:**271-281, 2004
10. Vail DM, Thamm DH: Mast cell tumors. *In:* Small Animal Clinical Oncology, eds. Withrow SJ, MacEwan EG, 4th ed., pp. 402-424. WB Saunders Co., 1997

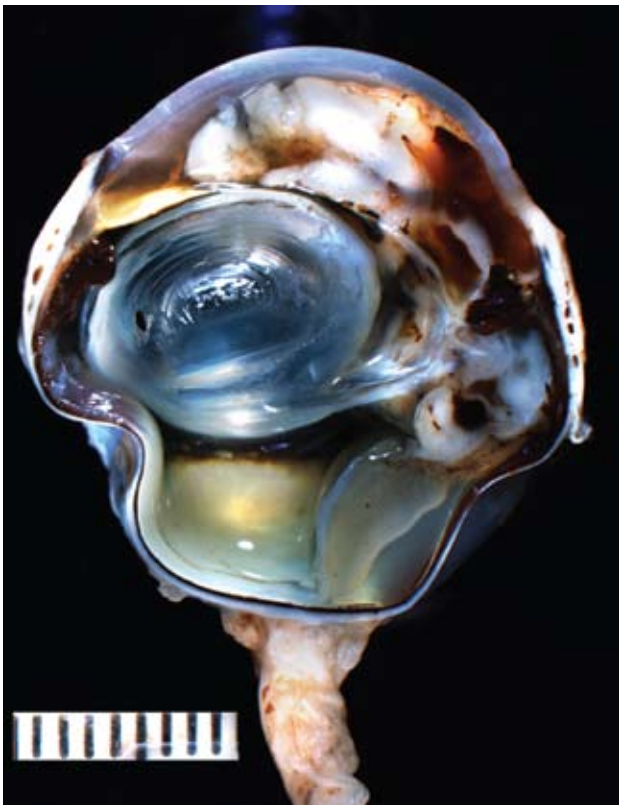
CASE III: 09RD990 (AFIP 3134608).

Signalment: 3-year-old spayed, female domestic shorthair cat (*Felis catus*).

History: The cat presented to a veterinary ophthalmologist 4 months prior to enucleation with an intraocular mass that involved approximately 25% of the ventral iris, extending from 4 o'clock to 7 o'clock. At the time of enucleation, the mass filled approximately 85% of the anterior chamber. There was no history of glaucoma.

Gross Pathology: Grossly, approximately 75% of the iris and anterior chamber are obliterated by a multinodular mass (**fig. 3-1**). On cut section, a solid white to tan mass bulges into the anterior chamber, fills the posterior chamber, and protrudes into the anterior vitreal space (**fig. 3-2**). The lens is displaced peripherally by the mass. Part of the retina is wrinkled and separated from the choroid with a translucent, gelatinous exudate in the subretinal space.

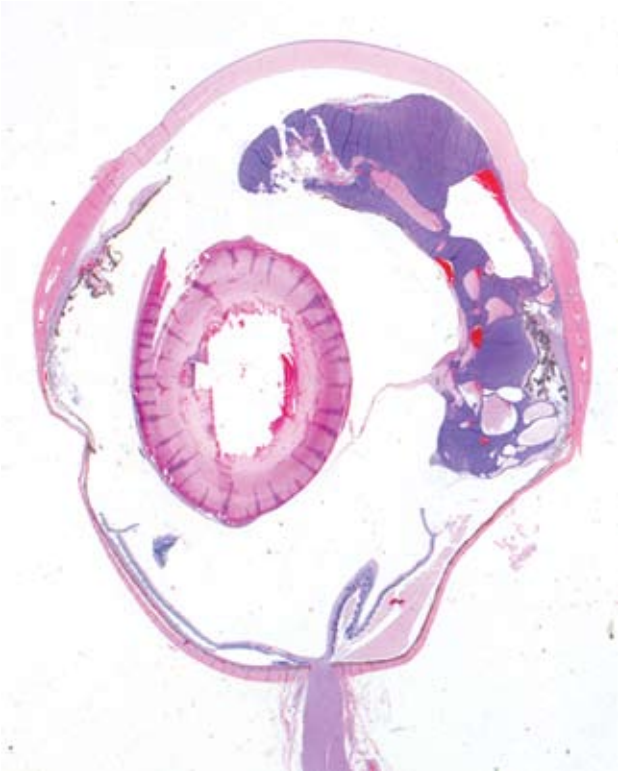




3-1, 3-2. Eye, cat, iridociliary adenoma. Photographs courtesy of Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, 2015 Linden Drive, Madison, WI 53701-1102, dubielzr@svm.vetmed.wisc.edu.

Histopathologic Description: Microscopically, there is a cross-section of globe with a mass that is broadly adherent to and continuous with the nonpigmented ciliary body epithelium and effaces the stroma of the iris leaflet and fills the iridocorneal angle (**fig. 3-3**). The mass is composed of tightly packed sheets or compressed cords of uniform polygonal cells with indistinct borders and scant cytoplasm, supported by a very fine vascular stroma. Neoplastic cells occasionally form rosettes and pseudorosettes (**figs. 3-4 and 3-5**). Nuclei are round to oval with a granular chromatin pattern and occasional single central nucleolus. There is moderate anisokaryosis and mitotic figures are rare (1 seen in ten 400x fields). There are several variably-sized cavitated spaces throughout the mass that contain blood and/or serum protein. At the periphery of the mass adjacent to the ciliary body, there is a rim of homogenous eosinophilic material that histologically resembles osteoid. The superior iridocorneal angle shows an open cleft and intact corneoscleral trabecular meshwork. The non-pigment ciliary body epithelium of the pars plana is expanded by multiple variably sized cysts. At the lens equator nearest to and presumed to have been in contact with the mass, there is degeneration and dissolution of the lens cortex characterized by spherical to irregularly-shaped eosinophilic aggregates of lens protein (Morgagnian globules), some of which contain a nucleus (bladder cells). The lens epithelium extends across the posterior aspect of the lens (subcapsular cataract). On the ventral aspect of the cornea, the mass abuts Descemet's membrane, the endothelium is unapparent and there is mild neovascularization of the superficial corneal stroma. Segmentally within the inferior retina, there is hypertrophy of the retinal pigment epithelium and retinal detachment with serum protein in the subretinal space. Ganglion cells are present in adequate numbers throughout the retina. Equivocally, there are increased numbers of glial cells within the substance of the optic nerve immediately posterior to the lamina cribrosa. An alcian blue periodic acid-Schiff (PAS) stain reveals a complex network of delicate PAS positive basement membranes surrounding individual or groups of neoplastic cells (**fig. 3-6**). Multifocally, both near and distant from the tumor, there is strandy to glassy Alcian blue positive material coating the inner aspect of the nonpigmented ciliary body epithelium (acid mucopolysaccharide secretion).

Contributor's Morphologic Diagnosis: 1. Feline iridociliary adenoma
2. Equatorial cortical cataract
3. Segmental retinal detachment
4. Epithelial cysts of the pars plana



3-3. Eye, cat, iridociliary adenoma. A highly cellular mass effaces a portion of the anterior uveal tract and is composed of sheets of small, uniform epithelial cells with several cavitated spaces variably filled with blood or serum protein. There is a focal area of retinal detachment with a protein exudate in the subretinal space. Photomicrograph courtesy of Department of Pathobiological Sciences, School of veterinary medicine, University of Wisconsin-Madison, 2015 Linden Drive, Madison, WI 53701-1102, dubielzr@svm.vetmed.wisc.edu.

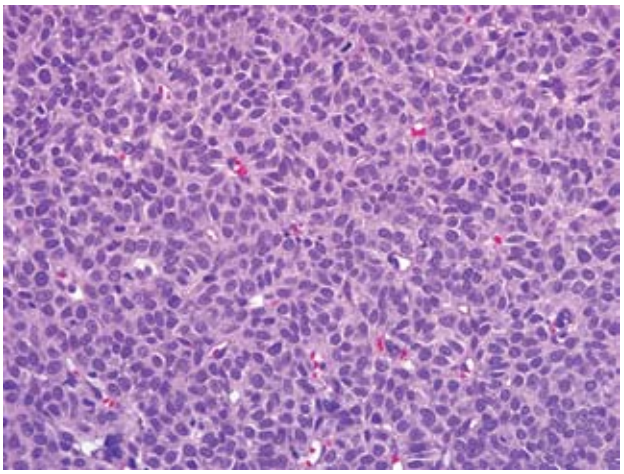
Contributor's Comment: In the slides submitted, the lens was removed for processing. This case was chosen because it represents the classic features of feline iridociliary adenoma.

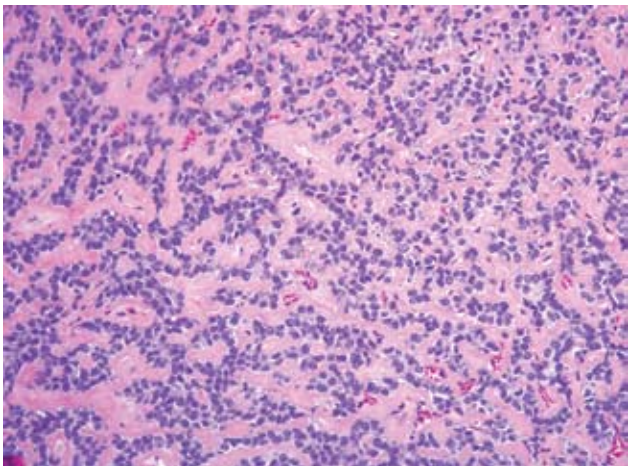
The epithelium of the iris and ciliary body is derived from the neuroectoderm by expanding forward from the margins of the optic cup.[2] Neoplasia of the neuroectoderm of animals is considered uncommon (iridociliary adenomas and carcinomas) or rare (medulloepitheliomas, retinoblastomas, astrocytomas and gliomas).[3,4] With the exception of ciliary adenocarcinomas, these neoplasia are considered benign.[3] Iridociliary epithelial tumors are the second and third most common primary intraocular tumor in the dog and cat, respectively.[1] They arise from the pigmented or non-pigmented epithelial cells, usually from the *pars plicata* of the ciliary body.[1]

Clinically, these tumors usually present as white to brown or black, fairly well delineated, sometimes pedunculated, slow growing masses, that usually grow into the vitreous behind the lens.[4] They are usually visible though the pupil in the posterior chamber. Invasion through the iris or protrusion through the pupil can lead to a localized mass visible in the anterior chamber.[3] Clinical signs usually include a retro-iridal mass that may displace the iris or lens by expansive growth, and if the tumor is large, secondary glaucoma, ocular pain and/or intraocular hemorrhage may be noted.[4] Hyphema and aqueous flare may be present due to the common formation of preiridal fibrovascular membranes.[3] In cases where the intraocular hemorrhage and flare impair the direct observation of the tumor, ultrasonographic imaging may prove helpful in delineating a mass lesion in the posterior chamber.[1]

Histologic features of feline iridociliary adenomas include a solid non-pigmented epithelial tumor with packets of cells surrounded by thin PAS positive basement membrane structures.[2] The defining feature of adenocarcinoma, versus adenoma, is invasion into the sclera by the neoplastic population rather than features of cellular atypia.[2] Thus, most iridociliary adenocarcinomas in both cats and dogs have relatively benign cellular features.

A retrospective study of 101 cases of feline iridociliary tumors conduced in the Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW) (unpublished data) identified other common features of these tumors. Of the 101 cases, 84 (83.1%) of the tumors were classified as adenomas. Seventeen (16.9%) presented scleral or choroidal invasion and were classified as adenocarcinomas. Adenomas presented a solid pattern in 97.6% (82/84) of the cases. They were either classified as uveoinvasive (52.3%) and non-uveoinvasive (47.7%). The most common histological features of adenomas were PAS positive basement membranes (present in 82.3% of the cases), cavitated spaces filled with blood and/or proteinaceous material (77.3%), presence of osteoid matrix (42.8%), osseous metaplasia (12%), and formation of pseudo-rosettes (26.2%). Mitotic figures were rare in both adenomas and adenocarcinomas. Two cases of iridociliary adenomas presented atypical patterns, one papillary and the other a mucoid variant that presents PAS positive basement membrane and positivity for vimentin and cytokeratin. Most tumors were positive for vimentin and only carcinomas presented variable positivity for cytokeratin. A previous study demonstrated that 50% of feline and canine iridociliary adenomas were positive for S-100, 1/3 of the feline cases were positive for glial fibrillary acidic protein (GFAP) and all canine cases were positive for neuron-specific enolase (NSE).[2]



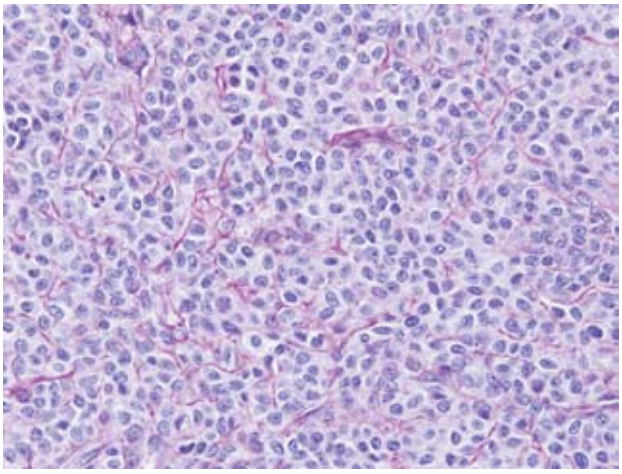


3-4, 3-5. Eye, cat, iridociliary adenoma. Markedly expanding the iris and ciliary body is a densely cellular neoplasm arranged in pseudorosettes, cords and tubules. (HE 400X)

In the recent unpublished study, the age of the animals ranged from 2 to 18 years (average 9.1). The most common secondary abnormalities detected were glaucoma, preiridal fibrovascular membrane, hyphema and retinal detachment. Presence of an intraocular mass was the main reason for presentation to the veterinarian and enucleation. Glaucoma is a common secondary condition associated with iridociliary tumors, in part because these tumors are associated with the formation of fibrovascular membranes that can lead to peripheral anterior synechia and obstruction of the iridocorneal angle.[1]

In the case presented, the evidence of glaucoma is equivocal. The most reliable microscopic evidence of glaucoma is absence or reduced numbers of retinal ganglion cells. In cats, even cases of chronic glaucoma can show an otherwise robust retina that lacks ganglion cells. In contrast, the glaucomatous canine globe frequently shows atrophy of the inner retinal layers to full-thickness atrophy in chronic cases. Gliosis of the optic nerve is also reliable evidence of glaucoma. Atrophy of the optic nerve with cupping of the optic nerve head is also common, particularly in dogs. Finally, a feature of glaucoma seen characteristically in dogs with primary glaucoma (goniodysgenesis) is "tapetal sparing" in which the tapetal (superior) retina is less profoundly atrophied than the non-tapetal (inferior) retina. Presence of epithelial cysts of the pars plana is a common finding in older cats and presumed to be a degenerative condition not related to the tumor in this case.

In sum, solid nonpigmented tumors arising from the ciliary body or iris epithelium with small epithelial cells packed by thin PAS-positive membranes and staining positive for vimentin are significant features defining iridociliary tumors in cats.



3-6. Eye, cat, iridociliary adenoma. PAS-positive basement membranes surround individual or clusters of neoplastic epithelial cells. (Alcian blue-PAS 200X) Photomicrograph courtesy of Department of Pathobiological Sciences, School of veterinary medicine, University of Wisconsin-Madison, 2015 Linden Drive, Madison, WI 53701-1102, dubielzr@svm.vetmed.wisc.edu.

AFIP Diagnosis: 1. Eye: Iridociliary adenoma.
2. Eye, retina: Detachment, segmental.

Conference Comment: We thank the contributor for providing this excellent example and thorough analysis of the entity. Conference participants discussed the microscopic features that distinguish iridociliary adenoma from adenocarcinoma, with emphasis on scleral invasion as an important feature of the latter, as the contributor

noted. While metastasis of iridociliary adenocarcinoma is extremely rare, it has been reported in dogs.[5] Normal iridociliary epithelium and adenomas express vimentin, but not cytokeratin, while adenocarcinomas may express both vimentin and cytokeratin AE1/AE3.[2,5]

Contributor: Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, 2015 Linden Drive, Madison, WI 53701 http://www.vetmed.wisc.edu/home

References:

1. Dubielzig RR: Tumors of the eye. *In:* Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 739-754. Iowa State Press, Ames, IA, 2002
2. Dubielzig RR, Steinberg H, Garvin H, Deehr AJ, Fisher B: Iridociliary epithelial tumors in 100 dogs and 17 cats: a morphological study. Vet. Ophth **1**:223-231, 1998
3. Grahn BH, Peiffer RL: Fundamentals of veterinary ophthalmic pathology. *In:* Veterinary Ophthalmology, ed. Gellat KN, 4th ed., pp. 355-437. Blackwell Publishing, Ames, IA, 2007
4. Miller PE, Dubielzig RR: Ocular tumors. *In:* Withrow & McEwen's Small Animal Clinical Oncology, ed. Withrow SJ, Vail, DM, 4th ed., pp. 686-698. Elsevier, St. Louis, MO, 2007
5. Zarfoss MK, Dubielzig RR: Metastatic iridociliary adenocarcinoma in a Labrador retriever. Vet Pathol **44**:672-676, 2007

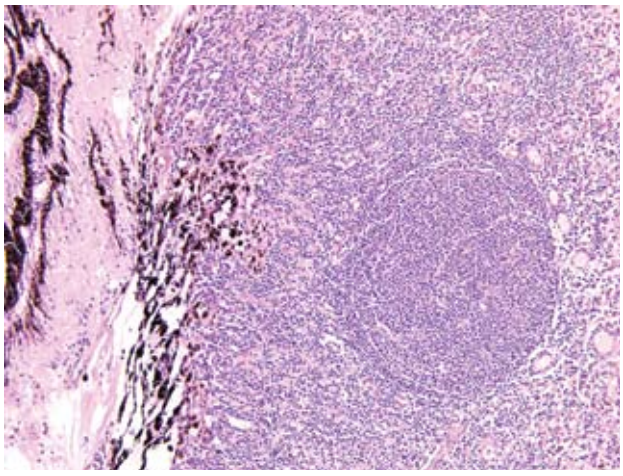
CASE IV: 09H2862 (AFIP 3134618).

Signalment: 7-year-old castrated, male paint horse (*Equus caballus*).

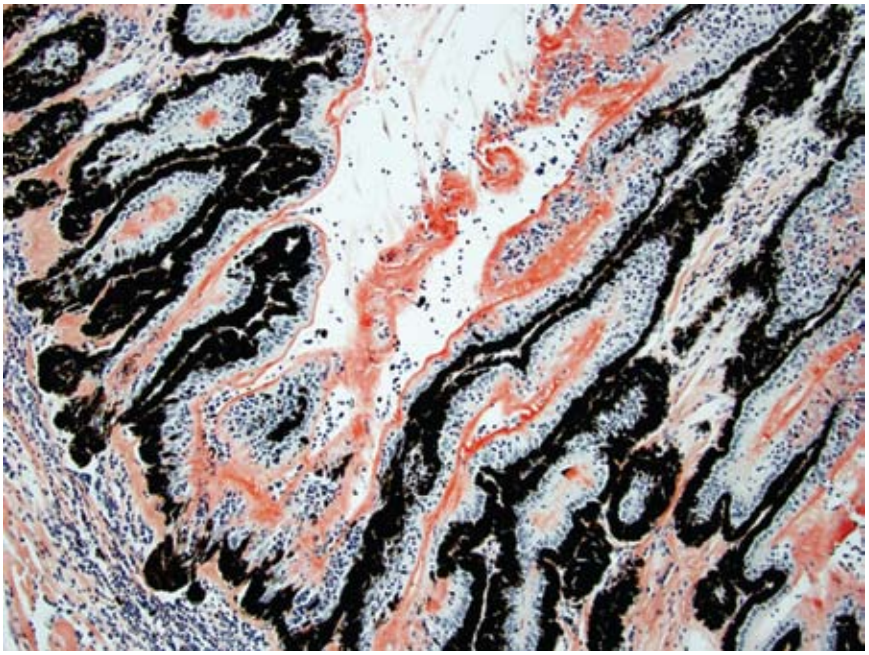
History: Small, non-visual left eye for the past one year; clinically suspected chronic uveitis. The right eye was normal.

Gross Pathology: Globe was somewhat small with a cloudy cornea.

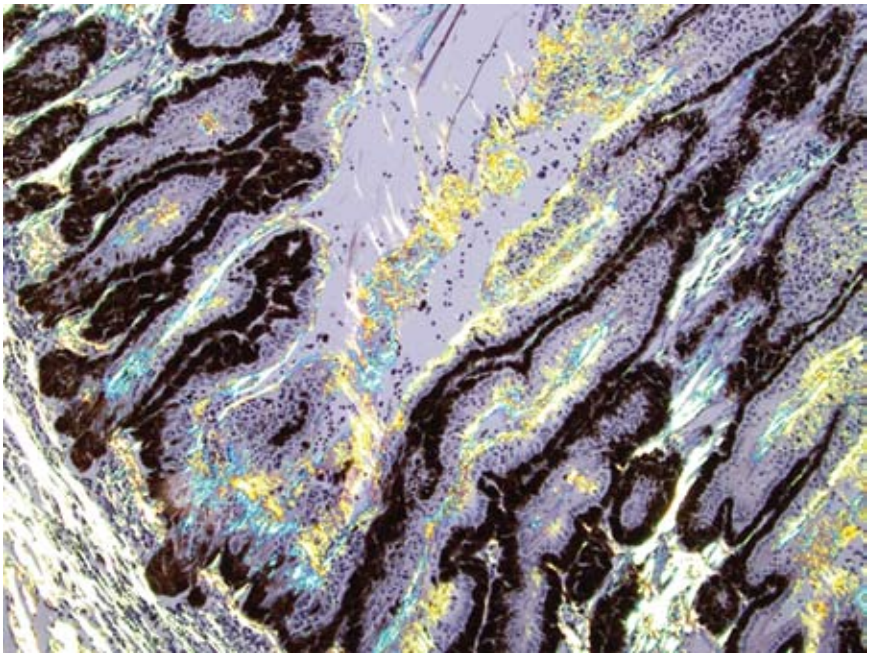
Histopathologic Description: Eyeball (a-d): The cornea contains a modest amount of neovasculature within the superficial third of the stroma. The anterior chamber is filled with eosinophilic flocculent material (serous exudate). The iris is greatly thickened and distorted; it contains a marked perivascular to diffuse infiltrate of plasma cells and lymphocytes (**fig. 4-1**). In addition, the anterior and posterior surfaces are covered with a layer of fibrovascular tissue (preiridial fibrovascular membrane) and adherent plasma cells and lymphocytes, which spans the pupil and is adhered to the anterior lens capsule (posterior synechia). The filtration angle is variably collapsed and the ciliary cleft contains a moderate accumulation of lymphocytes, plasma cells, and melanophages. The ciliary body is distorted and contains an infiltrate similar to the iris; in addition, it contains scattered follicular aggregates of lymphocytes and plasma cells. Also, the neuroepithelium of the ciliary body is covered by a thick layer of homogeneous eosinophilic, congophilic material that has a somewhat ragged surface; this material has green birefringence with polarized light (**figs. 4-2 and 4-3**). There is a layer of fibrillar fibrous material extending from the surface of the ciliary body along the surface of the posterior lens capsule (cyclitic membrane). The choroid is diffusely congested and contains a few scattered aggregates of lymphocytes and plasma cells. The retina is detached in several places and the subretinal space contains eosinophilic flocculent material and a few melanophages. The retinal pigment epithelium is hypertrophied in the corresponding detached areas. The nontapetal retina is thin due to a thinned nerve fiber layer, thinned inner and outer plexiform layers, a diminished number of ganglion cells, and a hypocellular inner nuclear layer, which somewhat blends into the outer nuclear layer. Ganglion cells that are present have central chromatolysis. The photoreceptors are evident but blunted. There are a few folds in the retina. The tapetal retina is more nearly normal. The lens capsule is ruptured in several foci, especially on the anterior surface adjacent



4-1. Eye, horse. Multifocally expanding the iris are nodular cellular infiltrates composed of lymphocytes and plasma cells. (HE 100X)



4-2. Eye, horse. Neuroepithelium of the ciliary body is covered by a thick layer of homogeneous eosinophilic amyloid-like material. (Congo red 100X) Photomicrograph courtesy of Department of Veterinary Pathology, Iowa State University, Ames, IA 50011, rkmyers@iastate.edu



4-3. Eye, horse. Homogeneous eosinophilic amyloid-like material, congo red positive material has green birefringence with polarized light. (Congo red, polarized, 100X) Photomicrograph courtesy of Department of Veterinary Pathology, Iowa State University, Ames, IA 50011, rkmyers@iastate.edu

to the posterior synechiae. In these areas, the anterior cortical lens fibers are swollen, fragmented and some have undergone fibrous metaplasia, which has produced a thick fibrous layer that covers the inner surface of the anterior lens capsule. The vitreous body has deteriorated and the vitreous chamber is filled with eosinophilic flocculent material (plasmoid vitreous). The bulbar conjunctiva at the limbus contains a marked follicular infiltrate of lymphocytes and plasma cells.

Contributor's Morphologic Diagnosis: Eyeball: Chronic lymphoplasmacytic uveitis (especially anterior uveitis) with posterior synechiae, partial filtration angle closure, cortical cataract with capsule rupture and fibrous metaplasia, retinal detachment and retinal degeneration, non-tapetal retina.

Contributor's Comment: This eye has chronic lymphoplasmacytic uveitis (especially anterior uveitis)

with many secondary changes that include posterior synechiae, partial filtration angle closure, cortical cataract, retinal detachment and retinal degeneration. The pattern of the uveitis with the accumulation of congophilic material (amyloid) on the ciliary body is typical of equine recurrent uveitis (ERU). ERU is a fairly common form of uveitis in horses; apparently there is breed predisposition, with Appaloosas being very susceptible and, when affected, having bilateral involvement about 80% of the time. Some aspects of the cause and pathogenesis are not clear, but the consensus seems to be that the uveitis develops due to an autoimmune reaction. The prevailing theory is that certain bacterial antigens, most notably those on certain serovars of *Leptospira interrogans* (especially *L. pomona*) are similar to intrinsic antigens in the uvea, and that the immune response generated against the bacterial antigens cross-reacts with the uveal antigens resulting in autoimmune mediated inflammation.[1] The retinal degeneration has a pattern that is consistent with secondary glaucoma (non-tapetal involvement) but also could have been caused by ocular hypertension, a condition that often accompanies ERU.[1]

AFIP Diagnosis: Eye: Endophthalmitis, lympho–plasmacytic, chronic, diffuse, mild to moderate, with posterior synechia, pre-iridial fibrovascular membrane, post-iridial amyloid-like material, retinal detachment and atrophy, peripheral corneal neovascularization, and cataractous change.

Conference Comment: Equine recurrent uveitis (ERU) is one of several immune-mediated endophthalmitides of veterinary importance; others include canine uveodermatologic syndrome, canine lymphocytic uveitis, feline idiopathic lymphonodular uveitis, and lens-induced uveitis. Because ERU is a progressive disease, a wide range of potential microscopic findings, from uveitis to endophthalmitis to phthisis bulbi, exists.[2] This case nicely illustrates several of the characteristic features of ERU, including the hallmark lesion, deposition of hyalinized congophilic (i.e. amyloid-like) material in the apical cytoplasm of the posterior iridociliary epithelium.[2] The presence of numerous additional pathologic findings makes for a very descriptively challenging slide. The retinal lesions, as suggested by the contributor, may be the result of secondary glaucoma; however, glaucoma is a rare complication of ERU, and it is thought that the trabecular meshwork is less important than uveoscleral resorption for aqueous drainage in horses, in contrast to the mechanism of aqueous outflow in dogs and cats.[1,2] Some researchers suggest that a retinopathy may be the primary immunological event in equine uveitis and that interphotoreceptor retinoid-binding protein (IRBP) is the major autoantigen in ERU. This autoantigen, along with others, may be unmasked by a variety of intraocular inflammatory events leading to a common final pathway that is characteristic of ERU. Alternatively, as alluded to by the contributor, the mimicry of autoantigens by infectious agents is a popular hypothesis; a 90-kd protein common to several serovars of *Leptospira interrogans* shares epitopes with an equine corneal peptide, lending credence to this hypothesis.[1]

The pathogenesis of immune-mediated endophthalmitis is complex and its discussion is enhanced by a rudimentary understanding of a recently-discovered, carefully-regulated system of ocular immunity called anterior chamber-associated immune deviation (ACAID). Briefly, ACAID is a series of responses to intraocular antigen that ultimately results in impaired delayed-type hypersensitivity (DTH) expression and suppressed complement-fixing antibody production without suppressing antigen-specific cytotoxic T cell activity. As a result, "innocent bystander" intraocular tissues are spared collateral damage from T cell-driven inflammation.[1] More specifically, a number of cytokines, e.g. TGF-β2, TNF-α, vasoactive intestinal peptide, and substance P, alter intraocular antigens, which are then processed by resident antigen presenting cells (APCs), i.e. macrophages and dendritic cells, within the uvea. The APCs travel to the spleen where they promote the development of specific suppressor T-cells, which then return to the uvea and suppress DTH and complement-fixing antibody production, but not cytotoxic T cell activity. When this system is overwhelmed or impaired by uncontrolled introduction of antigen into the globe, perivascular aggregates of lymphocytes develop in the iris, ciliary body, choroid, and retina, and may eventually form vague lymphoid follicles. Amplification results in decreased specificity for the initial inciting antigen among the acquired lymphoid population and a stereotyped response to circulating antigens that enter the eye through a now-damaged blood-eye barrier.[2]

Taken together, the evidence suggests that ERU is multifactorial, as is the predisposition to its development. The contributor mentioned that Appaloosas are predisposed, with 80% of cases being bilateral. In contrast, only 20% of non-Appaloosas with ERU develop bilateral disease, and Appaloosas with light coats and many spots are more likely to develop ERU than are Appaloosas with dark coats and few spots, suggesting that melanin may be protective. In German Warmblood horses, the MHC I haplotype ELA-A9 is strongly associated with ERU, further supporting the hypothesis of a genetic predisposition.[1]

Contributor: Department of Veterinary Pathology, Iowa State University, Ames, IA 50011
http://vetmed.iastate.edu/

References:

1. Brooks DE, Matthews AG: Equine ophthalmology. *In:* Veterinary Ophthalmology, ed. Gellat KN, 4th ed., pp. 1239, 1244-1248. Blackwell Publishing, Ames, IA, 2007
2. Wilcock BP: Eye and ear. *In:* Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 505-509. Elsevier Saunders, Philadelphia, PA, 2007