Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2011-2012

Conference 4

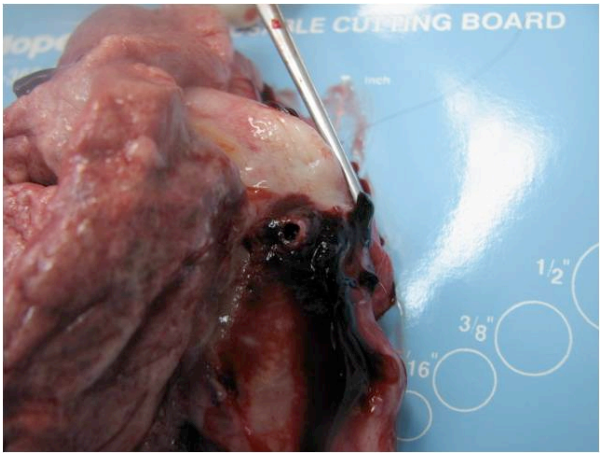
28 September 2011

CASE I: YN11-7424 (JPC 4006042).

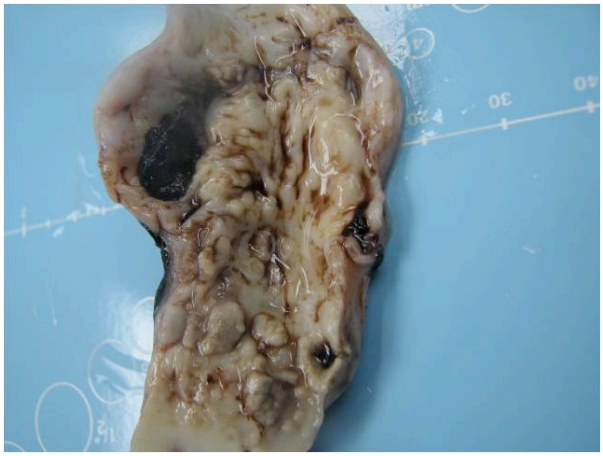
Signalment: 21-year-old female sooty mangabey (*Cercocebus atys*).

History: This 21-year-old female mangabey was born at the Yerkes National Primate Research Center and had been maintained in a social colony in an indoor–outdoor compound since birth. No significant clinical signs were reported in this animal and she had been observed eating and acting normally, approximately one hour prior to being found deceased in the indoor housing area at the Primate Center.

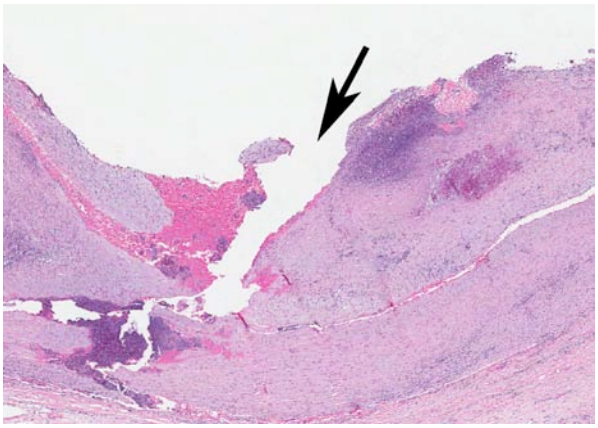
Gross Pathology: At necropsy, she weighed 7.25 kilograms and was in a good body condition. A large amount of clotted blood filled the left pleural space around the lung lobes. The wall of the thoracic aorta was moderately thickened and focally perforated (~0.15 cm diameter). The perforation had smooth edges and clotted blood was adhering to the tunica intima and adventitia. The tunica intima also had



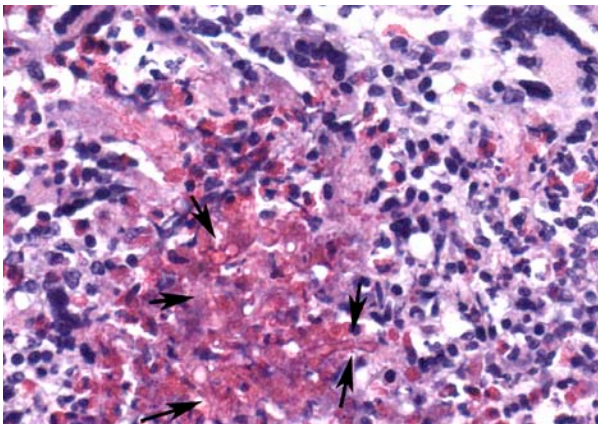
1-1. Aorta, sooty mangabey. The wall of the thoracic aorta is moderately thickened and focally perforated. Photograph courtesy of Yerkes National Primate Research Center, Atlanta, GA, www.yerkes.emory.edu/research/divisions/pathology.



1-2. Aorta, tunica intima, sooty mangabey. Within the tunica intima there are multiple firm yellow nodules. Photograph courtesy of Yerkes National Primate Research Center, Atlanta, GA, www.yerkes.emory.edu/research/divisions/pathology.



1-3. Aorta, sooty mangabey. The aortic wall is focally fractured and expanded by necrotic cellular debris. (HE 200X)



1-4. Aorta, sooty mangabey. Clusters of fungal hyphae with non-parallel walls and few septae incite a profound eosinophilic response. (arrow). In this case, silver stains for fungi were non-contributory. (HE 400X)

multiple, firm, pale yellow nodules (0.25cm-0.5 cm diameter).

Laboratory Results: PCR performed on the formalin-fixed paraffin-embedded aortic tissue confirmed *Basidiobolus* as the likely causative agent.

Histopathologic Description: Aorta: The aortic wall has moderate multifocal intimal fibrosis intermixed with necrosis and infiltrates of degenerate and viable eosinophils and neutrophils. The aortic intimal nodules contains pyogranulomatous inflammation intermixed with abundant eosinophils, necrosis, fibrin and broad, infrequently septate, thin-walled fungal hyphae, which occasionally show focal bulbous dilations and irregular branching. The immunohistochemistry for mucormycetes demonstrates fungal hyphae in areas of intense eosinophilic inflammation in the aortic intima. The inflammatory infiltrate often extends to the tunica media and occasionally to the tunica adventitia. No significant lesions or fungal hyphae were apparent in any other tissue.

Contributor's Morphologic Diagnosis: Aorta, severe multifocal pyogranulomatous to eosinophilic aortitis with intralesional fungal hyphae (*Basidiobolus* spp.)

Contributor's Comment: An aneurysm is a localized abnormal dilatation or outpouching of a thinned and weakened portion of a vessel, affecting usually large elastic arteries and hence can be fatal upon rupture.[8] Known causes of aneurysms in animals include copper deficiency in pigs, *Spirocerca lupi* infection in dogs and *Strongylus vulgaris* infection in horses.[8] In humans, aortic rupture can be spontaneous or a result of severe trauma, but in animals, physical trauma is a more common cause. Aortic rupture with dissecting aortic aneurysm has been studied in cattle due to similarities with Marfan syndrome in humans and it also occurs in ostrich, pigs, rats and turkeys.[8] Sudden rupture of the ascending aorta associated with marked exertion and excitement (due to breeding and racing) in horses has been associated with rapid death from cardiac tamponade.[8]

Aneurysm in the aortic arch associated with *Aspergillus* infection has been reported in a horse and fungal infections of the guttural pouch have been associated with ulceration of the internal carotid or maxillary artery resulting in their rupture and fatal hemorrhage in horses.[2,7] Though spontaneous aortic aneurysms have been described in NHPs along with dissecting aneurysms in monkeys used as experimental models of atherosclerosis[6], our case uniquely presents rupture of a spontaneous aortic aneurysm associated with entomophthoromycosis in a sooty mangabey. Immunohistochemistry detected a mucormycete infection in the aorta and the PCR further identified the fungus as *Basidiobolus* spp, a mucormycete.

Broadly, the term mucormycosis is the preferred name used to describe the angiotropic infection caused by a member of the subphylum *Mucoromycotina* or the subphylum *Entomophthoromycotina* (formerly Zygomycetes). Histopathology typically demonstrates angioinvasion with associated necrosis.[1] Medically important orders and genera include:

1. Mucorales, causing subcutaneous and systemic zygomycosis (Mucormycosis) - *Rhizopus, Lichtheimia (Absidia), Rhizomucor, Mucor, Cunninghamella, Saksenaea, Apophysomyces, Cokeromyces* and *Mortierella*
2. Entomophthorales, causing subcutaneous zygomycosis (Entomophthoromycosis) - *Conidiobolus* and *Basidiobolus.*[1]

Basidiobolus is a true pathogen, causing infections in immunocompetent and immunocompromised human

and animal hosts.[3] It occurs in decaying vegetation, soil, and as a saprobe in the intestinal contents of reptiles like lizards, chameleon, amphibians (toads) and mammals (bat).[3] The fungus is believed to enter the skin after insect bites, scratches and minor cuts. With the exception of *Basidiobolus ranarum,* the fungi in the Entomophthorales order, compared to those in Mucorales order, generally do not invade vascular tissue.[3] Tissue reaction to *Basidiobolus* infection may be acute with infiltrates of eosinophils, lymphocytes and plasma cells at the infected focus and/or a chronic granulomatous response with a predominance of eosinophils.

Finally, the aneurysm in the thoracic aorta of this sooty mangabey was a true aneurysm involving all the layers of the aortic wall and in the absence of any significant clinical signs and based on the gross and histologic lesions, the cause of death of this animal was an acute rupture of the aortic aneurysm associated with the fungal infection. The mucormycotic infection of the aorta was very severe and multifocal, but no evidence of a widespread infection of other organs was observed.

JPC Diagnosis: Aorta: Arteritis, proliferative, pyogranulomatous and eosinophilic, chronic-active, multifocal to coalescing, severe, with numerous fungal hyphae.

Conference Comment: Microscopically, aneurysms are either characterized by complete or partial rupture of the intima and media in the grossly stretched region, developing gradually with replacement by fibrous tissue, or, in the case dissecting or false aneurysms, fracture and necrosis in the media with dissection by blood. Neither of these lesions were present in the slide, so it is unclear if the aneurysm was caused by the fungal infection, or if it was merely secondarily colonized.[4]

Zygomycetes are ubiquitous, saprophytic, non-pigmented, 7-10 µm wide fungi with non-dichotomous branching and rare septa. The *Mucorales* order is more commonly pathogenic in warm-blooded animals, with angioinvasion being characteristic, while the *Entomophthorales* order, which usually infects arthropods, reptiles, and amphibians, is rarely pathogenic to warm-blooded animals. Recent taxonomic restructuring combines the previous four species of the genus *Basidiobolus* into the single species, *B. ranarum.* The typical mode of transmition of *Basidiobolus* and other *Entomophthorales* is via ingestion of insects, which carry the spores on their external bristles, by reptiles and amphibians, which then pass infective spores in their excreta.[11]

Differential diagnoses for these fungal hyphae should include *Pythium* and *Lagenidium,* members of the *Oomycetes* class.[11] Polymerase chain reaction is needed to definitively differentiate these three pathogens. Another differential diagnosis that should be considered is the proliferative arteriopathy associated with SIV infection. With SIV, large to medium vessels are typically affected, particularly pulmonary arteries. The intima and media are thickened, the internal elastic lamina is fragmented, and the endothelium can be hypertrophied or hyperplastic, or focally eroded with adherent thrombi. Eosinophilic and granulomatous inflammation, as seen in this case, is typically not seen.[5]

Some conference participants reported seeing Warthin-Finkeldey-type syncytial cells within germinal centers of the associated hyperplastic lymph node, which are occasionally seen in non-human primates infected with simian immunodeficiency virus (SIV) or measles virus, and represent the fusion of infected and uninfected CD4+ T lymphocytes. These cells have also seen in humans infected with HIV.[9] Attempts to characterize these syncitial cells through immunohistochemistry and electron microscopy have been unfruitful, but they may represent a multinucleated follicular dendritic cell.[10] While the presence of multinucleated cells was confirmed in the lymph node, the contributor confirmed that this sooty mangabey was seronegative for SIV.

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CASE II: 10L2130 (JPC 4006056).

Signalment: Adult male Chukar partridge, *Alectoris chukar.*

History: Farm raised chukar, found dead.

Gross Pathology: Hepatic necrosis, multifocal, severe. Typhlitis, necrotizing, multifocal, severe. Nematodiasis, small intestine and cecae, severe.

Histopathologic Description: Liver: Multifocal, variably sized, random areas of coagulative and lytic necrosis replace hepatocytes throughout all lobules. Large numbers of macrophages, degenerative and intact heterophils intermixed with foreign body-type multinucleated giant cells are adjacent to areas of necrosis. Fewer lymphocytes and plasma cells surround these areas. Large numbers of macrophages and multinucleated giant cells contain myriads of intracytoplasmic, approximately 15 µm diameter, round, lightly eosinophilic protozoal trophozoites. Cecum: Similar trophozoites fill macrophages and multinucleated giant cells surrounded by degenerative heterophils, karyorrhectic debris and proteinaceous material cover the ulcerated lamina propria.

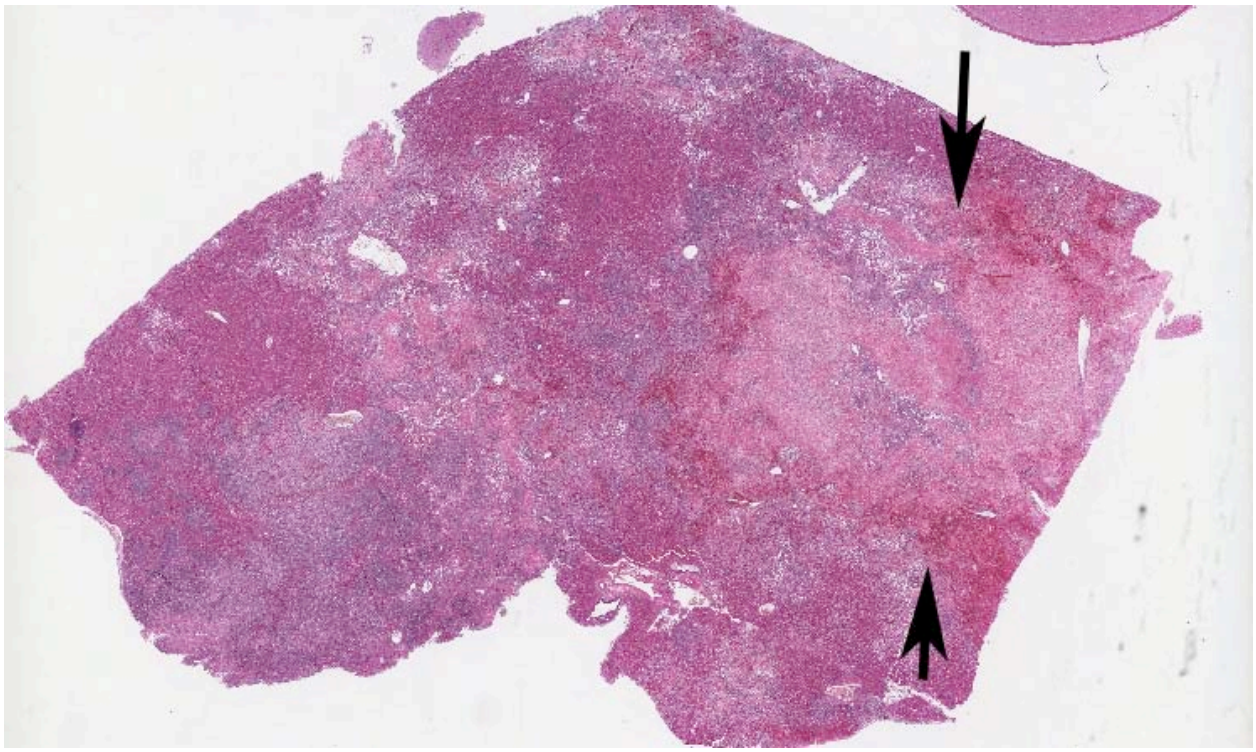
Contributor's Morphologic Diagnosis: Hepatocellular necrosis, lymphocytic-histiocytic hepatitis, with intracellular and intralesional trophozoites consistent with *Histomonas meleagridis.*

Typhlitis, necrotizing, multifocal, severe, and histiocytic, with intrahistiocytic and intralesional trophozoites consistent with *Histomonas meleagridis.*

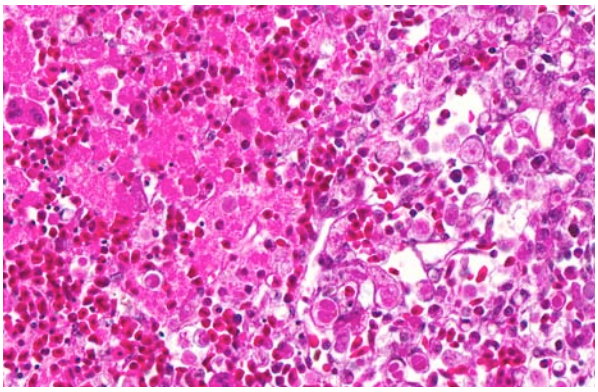
Contributor's Comment: Histomoniasis is caused by a flagellated protozoan parasite, *Histomonas meleagridis*, that colonizes earthworms and the cecal nematode, *Heterakis gallinarum.*[1,2] Histomoniasis primarily affects gallinaceous birds (chickens, grouse, partridge, peafowl, pheasants, quail, turkeys); lesions are primarily found within the liver and cecum.[5,6] Turkeys, either wild or domestic, almost always develop severe disease following infection. Chukar partridge, peafowl and ruffed grouse also are prone to severe disease.[4.] Ring-necked pheasants, chickens and junglefowl rarely become sick; these species serve as carriers of the parasite. Bobwhites, guinea fowl and Hungarian partridge exhibit high morbidity, but intermediate mortality.

JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal to coalescing, severe, with numerous protozoal trophozoites.
Brain: No significant lesions.

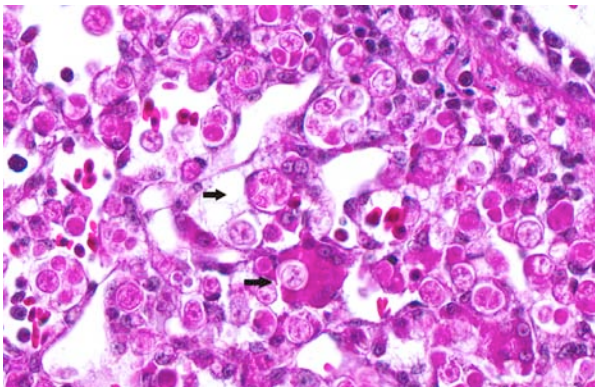
Conference Comment: This historically important disease has caused more losses to the turkey industry than any other disease, and occurs especially in poults, as well as chickens and captive game birds. Although the disease is less severe in chickens, economic losses are often greater than in turkeys due to the frequency



2-1 Liver, chukar partridge. There are coalescing areas of pallor (coagulative necrosis – arrows) and hemorrhage throughout the liver. (HE, 63X)



2-2. Liver, chukar partridge. Numerous extra- and intracellular trophozoites (arrows) populate areas of necrosis, with a sharply demarcated area of coagulative necrosis at left. (HE 200X)



2-3. Liver, chukar partridge. Multinucleated giant cell macrophages containing trophozoites are scattered thoughout the section. (HE 400X)

of occurrence and greater number of birds affected.[7] *Histomonas* was originally classified as an amoeba, but was reclassified as a flagellated protozoan by Tyzzer in 1920 based on the presence of a flagellum when in the cecal lumen and absence of a cyst form.[6] Although histomoniasis is colloquially called "blackhead" for the development of cyanosis of the head, this inconsistent sign is not pathognomonic. The conversion of hemoglobin to methemoglobin in acute disease may also contribute to cyanosis.[7]

Transmission is by ingestion of 1) infected feces; 2) embryonated *Heterakis* eggs containing histomonads; or 3) an earthworm containing infected *Heterakis* larvae. Histomonads are released into the intestinal lumen when the *Heterakis* eggs hatch and then invade the cecal wall. Two to three days after cecal infection, the protozoa reach the liver via hepatic-portal circulation, and can also be found in the bursa of Fabricus, kidney, pancreas, and spleen.[7] In addition to vector-borne transmission, histomoniasis can spread directly through cloacal drinking, which is the retrograde peristalsis of urine and fecal contaminants from the vent into the bursa and ceca; this is important in rapid spread of the disease through turkey flocks.[6]

In addition to the gross lesions seen in this case, the ceca are often bilaterally enlarged and hyperemic with thickened walls, and may contain a central caseous, laminated core, necessitating differentiation from *Eimeria tenella* and *Salmonella* spp. The liver may have depressed targetoid lesions which often coalesce. *Histomonas* may be difficult to identify histologically in chronic lesions, and the presence of rounded empty spaces within a marked inflammatory response should raise the specter of histomoniasis, especially within the liver or ceca.[2]

Histomonas achieves its full virulent potential when *Escherichia coli*, *Clostridium perfringens*, or *Bacillus subtilis* is present in the cecum, and is avirulent in gnotobiotic poults. Conversely, *Histomonas* mitigates *Eimeria* infections in the cecum by creating an inhospitable environment for coccidia to flourish. In chickens with histomoniasis, coinfection with *Eimeria tenella* significantly increases the development of hepatic necrosis.[7]

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References:

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CASE III: 11-A-207 (JPC 4006289).

Signalment: 7 year and 346 day-old intact male rhesus macaque, *Macaca mulatta*.

History: This animal had a history of ill thrift and intermittent diarrhea. *Campylobacter coli and Campylobacter jejuni* were isolated on fecal culture several months prior to euthanasia. *Balantidium coli* and eggs of *Trichuris spp*. were identified on fecal parasitology screens at the same time. Supportive and therapeutic care was provided. Euthanasia was performed due to the poor prognosis for long-term resolution of diarrhea.

Gross Pathology: The animal was thin with no visible subcutaneous or visceral adipose tissue stores. The mesenteric lymph nodes were multifocally enlarged up to five times the normal size. The large intestine was markedly and uniformly dilated and contained green liquid fecal material admixed with gas. Numerous 2 cm long slender nematodes were present in the lumens that are consistent with *Trichuris* sp. The mucosa of the cecum, ascending colon and transverse colon was diffusely thickened, edematous and red.

Laboratory Results: Normal ranges are given in parentheses.

Albumin (g/dl):	2.4	(3.4 - 4.70)
Protein (g/dl):	5.5	(5.8 – 7.5)

Histopathologic Description: Colon: Diffusely, the colonic mucosa is thickened with a moderate inflammatory infiltrate composed of large numbers of neutrophils, plasma cells, lymphocytes and lesser numbers of eosinophils and macrophages that separate the crypts and often extend into the submucosa. Scattered within the mucosa are lesser numbers of large foamy macrophages measuring 8 - 20 µm in diameter and forming rare multinucleated giant cells containing up to 15 nuclei (muciphages). Crypt lumina multifocally contain intact and degenerate neutrophils admixed with cellular debris (crypt abscess) and affected crypts may be lined by attenuated epithelium. Occasionally the crypts are ruptured. Multifocally, there is mild loss of goblet cells. The remaining crypts are hypercellular and tall with moderate numbers of mitotic figures. The mucosal surface is multifocally eroded, irregular and attenuated with epithelial tags projecting into the lumen. Multifocally, the submucosal lymphatic vessels are dilated and contain eosinophilic flocculent fluid. Focally within the mucosa and within the lumen are 20-80 µm diameter protozoans with granular eosinophilic protoplasm containing variable numbers of vacuoles, a round to bean shaped nucleus, and a cell membrane covered with cilia. There are cross and tangential sections of nematodes with irregularly spaced projections on the cuticle, a stichosome surrounding the esophagus, a single prominent bacillary band, coelomyarian/ polymyarian musculature, pseudocoelom containing deeply eosinophilic fluid, digestive tract lined by uninucleate cells with a microvillous border, and ovary and uterus containing large numbers of thick shelled, oval unembryonated eggs with bipolar plugs. Multifocally, a wispy, basophilic mat of spirochetes is adhered to the microvillous border of the enteroocytes.

Contributor's Morphologic Diagnosis: Colon: Colitis, proliferative, neutrophilic, lymphoplasmacytic, eosinophilic, chronic-active, moderate, diffuse, with crypt abscesses, intralesional protozoa consistent with *Balantidium coli*, and intraluminal nematodes consistent with *Trichuris spp*.
Colon: Spirochetes, apical cytoplasm, moderate numbers, multifocal.
Microscopic findings of tissues not submitted:
Duodenum, jejunum and ileum: Enteritis, lymphoplasmacytic and eosinophilic, mild, multifocal with moderate deposits of amyloid in the lamina propria.
Spleen: Amyloid deposition, multifocal, mild.
Liver, space of Disse: Amyloid deposition, multifocal, minimal.
Adrenal gland, corticomedullary junction: Amyloid deposition, multifocal, minimal.

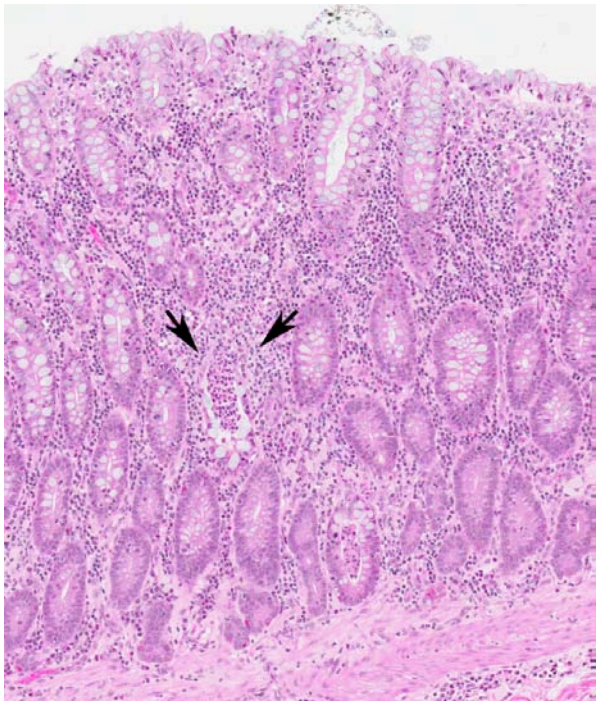
Contributor's Comment: The primary findings in this case are proliferative, neutrophilic, lymphoplasmacytic typhlocolitis with mesenteric lymphoid hyperplasia, and systemic amyloidosis. Chronic colitis in rhesus macaques is a complex syndrome with a prolonged clinical course of intermittent diarrhea, dehydration and poor growth rate. Juvenile to young animals are usually affected. The initiating and perpetuating cause of the chronic colitis is unknown and is most likely multifactorial. Microbial infection, stress, exposure to dietary antigens and inappropriate immune response are all thought to play a role in its pathogenesis.

Numerous enteric pathogens have been examined for their role in the development of chronic colitis. *C. coli* and *C. jejuni* were identified to be strongly associated with chronic colitis in rhesus macaques.[5] In a recent study, significant differences in the gastrointestinal tract microbial population have been reported between healthy animals and animals with chronic colitis.[5] In group housed colonies at ONPRC, *Campylobacter spp.* or *Shigella spp.* have been associated with initial episodes of diarrhea followed by presence of normal flora.[4] Virulent shiga toxin/eaeA intimin expressing *Escherichia coli, Balantidium coli, Giardia lamblia, Enterocytozoon bieneusi and Trichuris trichiura* have all been isolated in animals with and without diarrhea.[5]

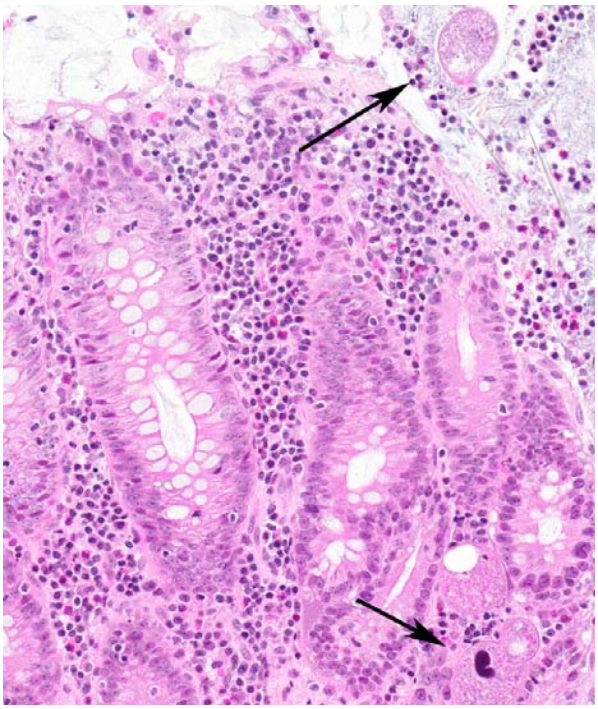
Grossly, most of the animals with chronic idiopathic colitis are emaciated with no subcutaneous or visceral adipose tissue stores, and mild to severe thymic atrophy.[1] The large intestine may be dilated and contain liquid fecal material. The cecum, ascending colon, and transverse colon are consistently affected. In severe cases, most of the large intestine may be involved; however, the rectum is seldom affected. The affected mucosa is thickened and may have a rugose appearance[1] with or without erosions or ulcerations. The mesenteric lymph nodes, ileocecal lymph nodes and colonic lymph nodes are usually hyperplastic. In severely affected animals, serous atrophy of visceral adipose tissue may be seen. Gastritis is not a consistent finding in animals with chronic idiopathic colitis.[6]

Microscopically, the colonic mucosa is thickened variably with neutrophils, lymphocytes, plasma cells that often separate the crypts. Crypt abscesses and crypt ruptures are common in acute infections.[1] In chronic cases, the mucosa is proliferative; the crypts may be distorted, tortuous with karyomegaly, hyperchromicity, pseudostratification, frequent mitotic figures and presence of mitotic figures in the upper 1/3 of the mucosal glands. Goblet cell loss is common. The epithelial changes include micro-erosions, attenuation, irregularities of cell shape and size, disparity of nuclear size and hyperchromicity. Mucosal herniation into the submucosal lymphoid tissue is common. The ileum may be involved variably. Other associated histological changes include lymphoid hyperplasia of mesenteric lymph nodes, chronic cholecystitis, mild portal lymphocytic hepatitis and thymic atrophy.[1] Chronic inflammation is a risk factor for developing reactive amyloidosis and chronic colitis is a common underlying inflammatory condition leading to the development of amyloidosis in this species. Amyloid deposition may be present in the lamina propria of small intestine, spleen, liver, adrenal gland and kidney.[2]

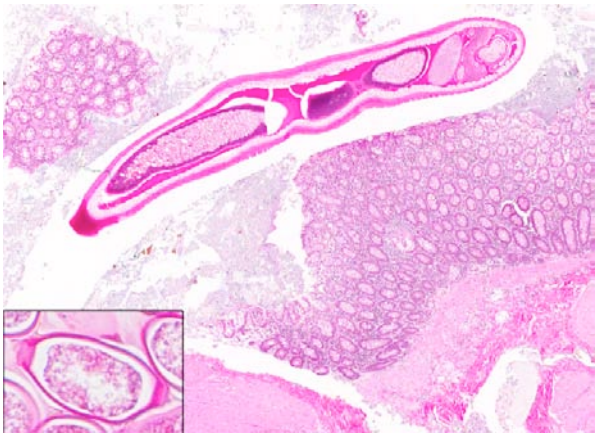
This case exhibits all the classic gross and histologic features of chronic colitis in macaques but has some additional features. Numerous protozoa are present within the mucosa as well as in the lumen and the nematodes are present within the lumen. Additionally, moderate numbers of foamy macrophages (muciphages) and multinucleated giant cells are present. Their significance is unknown. (Acid fast stains were performed to rule out possible *Mycobacteria*. sp.) The filamentous spriochetes visible in some portions of the section are relatively uncommonly seen in macaques with chronic diarrhea but are typically found in animals with normal colons. Spirochetes in the large intestine of rhesus macaques have not been associated with disease processes and are generally considered benign. In addition,



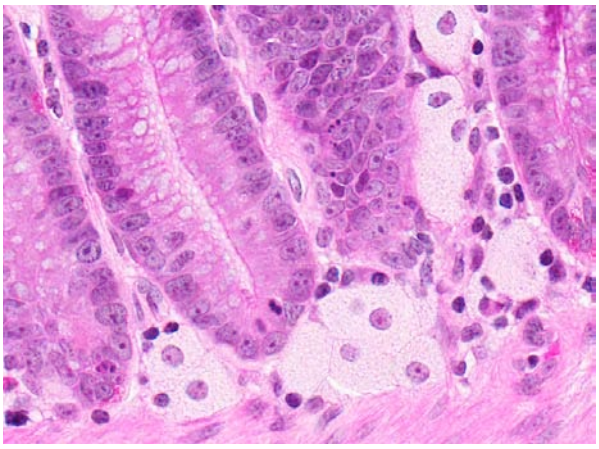
3-1. Colon, rhesus macaque. A profound lymphoplasmacytic inflammatory response in the colon separates and replaces colonic glands. There are numerous mitotic figures within the apical population, and a focal crypt abscess (arrows). "Crypt abscess" is a colloquial term for a dilated gland or crypt filled contain necrotic epithelial cells, neutrophils, and cellular debris. (HE 100X)



3-2. Colon, rhesus macaque. Luminal and invasive ciliates consistent with Balantidium coli. (HE 200X)



3-3. Colon, rhesus macaque. An adult nematode consistent with Trichuris trichiuria is present within colonic lumen. (HE 63X) The inset at lower left (400X) shows typical oval eggs with bipolar plugs.



3-4. Colon, rhesus macaque. Large macrophages with abundant foamy cytoplasm (muciphages) are presnt at the base of the glands, and in lesser numbers at the villar tips. A proposed reason for their presence is damage to goblet cells. (HE 400X)

Congophilic amyloid deposits were present in the lamina propria of duodenum, jejunum and, ileum as well as spleen, liver and adrenal glands. The gross and microscopic findings suggest that the severity of chronic diarrhea in this animal could be multifactorial, i.e., the presence of chronic colitis, balantidiasis, trichuriasis and enteric amyloidosis. Enteric amyloid deposition leading to protein-losing enteropathy accounts for the laboratory finding of panhypoproteinemia.

JPC Diagnosis: Colon: Colitis, proliferative, lymphoplasmacytic, chronic, diffuse, moderate, with crypt abscesses, luminal and intramucosal ciliates, and luminal adult aphasmid nematodes.

Conference Comment: The contributor has provided a comprehensive overview of chronic colitis in macaques. Chronic colitis of juvenile rhesus macaques, which typically occurs in animals from 10 months to three years of age, must be distinguished from chronic diarrhea from opportunistic infections associated with simian acquired immunodeficiency syndrome. Colonic neoplasia has not been associated with chronic colitis of juvenile rhesus macaques.[1] Conference participants also discussed the differential diagnosis of ulcerative cicatrizing colitis, which is also seen in rhesus macaques. In this disease, there is deep linear mucosal ulceration in the cecum and proximal colon, and resultant bands of reactive fibrosis cause colonic strictures.[4] This disease must be distinguished from colonic adenocarcinoma, a very common malignancy in older rhesus macaques, which often arises in the same location.

Muciphages are present in the superficial and deep mucosa and may be a response to damaged goblet cells. In the human, muciphages are seen in both normal and diseased large intestine and rectum, and are not considered predictive of disease.

The contributor mentioned the presence of mitotic figures in the upper 1/3 of the mucosal glands, and conference participants discussed this finding as a way to differentiate significant proliferative disease from normal mucosal epithelium turnover. Another helpful indicator of proliferative colitis is the relative paucity of goblet cells, as they are terminally differentiated cells and will not be present in newly regenerated epithelium. Chronic colitis of juvenile rhesus macaques differs from proliferative enteritides in other species in that severe ulceration and hematochezia, as well as the preneoplastic crypt mucosal dysplasia seen in the ulcerative diseases, are not present.

The spirochetes observed in some sections may be *Helicobacter cinaedi*, which has been associated with chronic colitis in a rhesus macaque, and has been shown to induce diarrhea and bacteremia in pigtail macaques.[3] There is superficial necrotizing colitis multifocally throughout this section, with neutrophilic and histiocytic inflammation, which is an indicator of active disease and is likely due to *Campylobacter jejuni* and *C. coli* overgrowth. These are usually seen in initial episodes, but *Campylobacter* and protozoans may be absent in subsequent episodes of the disease.[4]

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CASE IV: AFRRI Case 1 (JPC 4006476).

Signalment: 2-year-old spayed female domestic short hair cat.

History: On presentation the cat had a temperature of 105° F and was severely icteric.

Gross Pathology: Necropsy revealed minimal abdominal serocellular effusion, a pale swollen liver and shrunken, indented kidneys.

Laboratory Results:

Species	Breed	Sex	Pet Age	Reported
Feline	Domestic Short Hair	SF	1Y	4/23/11 1:51 PM

Test Requested	Results	Ref Range	Units
Superchem			
Total Protein	10.5 (HIGH)	5.2-8.8	g/dL
Albumin	LOW)	2.5-3.9	g/dL
Globulin	8.3 (HIGH)	2.3-5.3	g/dL
Albumin/ Globulin Ratio	0.3 (LOW)	0.35-1.5	Ratio
AST (SGOT)	44	10-100	U/L
ALT (SGPT)	52	10-100	U/L
Alk Phosphatase	51	6-102	U/L
GGTP	6	1-10	U/L
Total Bilirubin	0.5 (HIGH)	0.1-0.4	mg/dL
Urea Nitrogen	10 (LOW)	14-36	mg/dL
Creatinine	0.7	0.6-2.4	mg/dL
BUN/Creatinine Ratio	14	4-33	Ratio
Phosphorus	4.6	2.4-8.2	mg/dL
Glucose	110	64-170	mg/dL
Calcium	7.9 (LOW)	8.2-10.8	mg/dL
Magnesium	1.9	1.5-2.5	mEq/L
Sodium	142 (LOW)	145-158	mEq/L
Potassium	3.8	3.4-5.6	mEq/L
Na/K Ratio	37		
Chloride	111	104-128	mEq/L
Cholesterol	164	75-220	mg/dL
Triglycerides	77	25-160	mg/dL
Amylase	1017	100-1200	U/L
Lipase	42	0-205	U/L
CPK	157	56-529	U/L

CBC			
WBC	11.4	3.5-16.0	10^3/µL
RBC	6.77	5.92-9.93	10^6/µL
Hemoglobin	8.4 (LOW)	9.3-15.9	g/dL
Hematocrit	28.2 (LOW)	29-48	%
MCV	42	37-61	fL
MCH	12.4	11-21	pg
MCHC	29.8 (LOW)	30-38	g/dL
Platelet Count	139 (LOW)	200-500	10^3/µL
Platelet EST	Adequate	Adequate	

Platelet count reflects the minimum number due to platelet clumping.

Differential	Absolute	%	Ref Range	Units
Neutrophils	9348 (HIGH)	82	2500-8500	10^9/L
Bands	0	0	0-150	/µL
Lymphocytes	1596	14	1200-8000	
Monocytes	228	2	0-600	
Eosinophils	228	2	0-1000	
Basophils	0	0	0-150	

FeLV Antigen (ELISA)	Negative
FIV Antibody	**Positive**

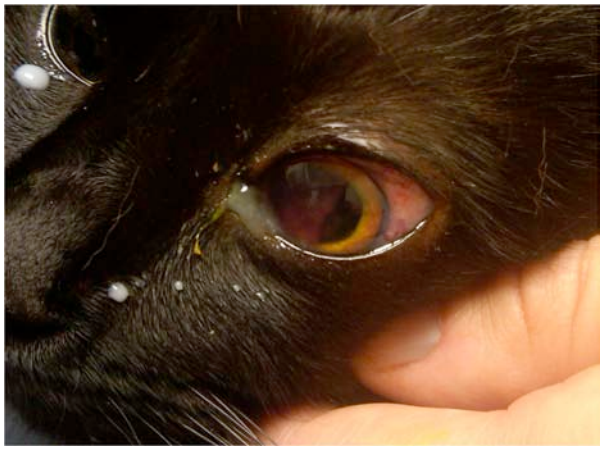
Result verified.
May be due to infection, vaccination, or (in kittens) maternal antibody.

FCV (IFA)	
1:400	**Positive**
1:1600	**Positive**

A positive FCV titer indicates exposure to a coronavirus. It does not differentiate between FIP, feline enteric coronavirus exposure, or vaccination. Diagnosis of FIP should be based on history, physical examination, and other laboratory findings, including electrophoresis on effusions. FIP PCR testing on effusions, and/or FIP 7b ELISA testing on serum may be helpful in confirming FIP infection.

Toxoplasma IgG Antibody	Negative
Toxoplasma IgM Antibody	

**********FELINE**********



4-1. Eye, cat. The cat presented with anterior uveitis, hyphema, and a mucopurulent ocular discharge. Photograph courtesy of Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-4712, http://www.usuhs.mil/afrri/.

IgM negative, IgG negative:
No serologic evidence of exposure to *Toxoplasma gondii*. However, some acutely infected cats are clinically ill prior to seroconversion. If clinical signs of toxoplasmosis are present, institute treatment and repeat serologic testing in 21 days to look for rising titers.

IgM 1:64 or greater, IgG negative or IgG 1:64 or greater:
Results are most consistent with recent exposure or active *Toxoplasma*
gondii infection. If clinical signs of Toxoplasmosis are present,

Urine Microalbumin (Feline)	Results	Ref Range	Units
Microalbuminuria	**12.2 (HIGH)**	**<2.5**	**mg/dL**

The MA is greater than 2.5 mg/dl and less than 30 mg/dl, indicating microalbuminuria.

institute treatment and repeat serologic testing in 21 days to look
for rising titers.

IgM>1:256, IgG negative or IgG 1:64 or greater:
Results are most consistent with active *Toxoplasma gondii* infection.

No serologic test documents clinical toxoplasmosis, but IgM titers >1:256 have almost exclusively been detected in clinically ill cats.

IgM negative, IgG 1:64 or greater:
Results are most consistent with chronic toxoplasmosis. However, IgM titers decrease rapidly in some infected cats. If clinical signs of Toxoplasmosis are present, institute treatment and repeat serologic testing in 21 days to look for rising titers.

Microalbuminuria (MA) usually indicates compromise of the glomerular barrier and is a significant finding when it is persistent (2 or more positive results obtained 2 or more weeks apart).

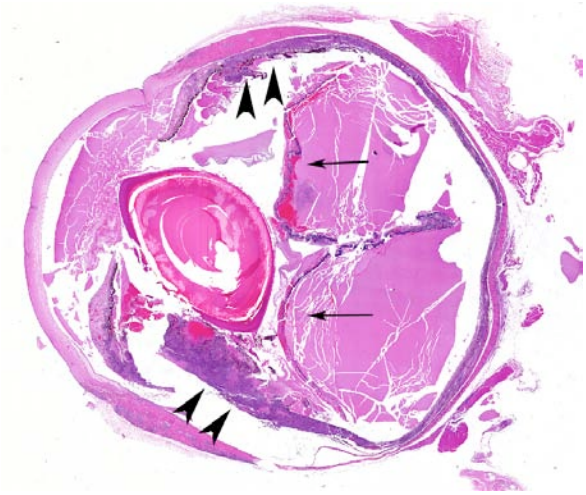
Persistent MA, in the majority of pets, is due to renal injury secondary to other systemic disease or primary renal disease. Systemic diseases associated with persistent MA include inflammatory disease, chronic infections, metabolic disease (e.g. hypertension, Cushing's Syndrome, diabetes mellitus, hyperthyroidism) and neoplasia. False positive results may occur with pyuria and gross hematuria. Suggestions for evaluating patients with microalbuminuria:

1. Check for and treat underlying diseases indicated above
2. Recheck MA in 2-4 weeks
3. In the absence of underlying disease, monitor for progression of MA and development of renal failure

Histopathologic Description: Eye and eyelid: Multifocal to coalescing, effacing and expanding 95%

Urinalysis	Results	Ref Range	Units
Collection Method	**Cystocentesis**		
Color	**Dark Yellow**		
Appearance	**Cloudy**	***Clear**	
Specific Gravity	**1.054**	**1.015-1.060**	
pH	**7.0**	**5.5-7.0**	
Protein	**2+ (HIGH)**	**Neg**	
Glucose	**Negative**	**Neg**	
Ketone	**Negative**	**Neg**	
Bilirubin	**2+ (HIGH)**	**Neg**	
Urine bilirubin verified by Ictotest.			
Blood	**Negative**	**Neg**	
WBC	**2-3**	**0-3**	**HPF**
RBC	**None**	**0-3**	**HPF**
Casts	**None Seen**		**LPF**
Struvite Crystals	**0-1**		**HPF**
Bacteria	**None Seen**	**None**	**HPF**
Squamous Epithelia	**0-1**	**0-3**	**HPF**

of the sclera and 75% of the ciliary body and iris are high numbers of neutrophils, macrophages, plasma cells and fewer lymphocytes, admixed with reactive fibroblasts, loose collagen, ectatic lymphatics with abundant pale eosinophilic proteinaceous material (edema), beaded to fibrillar material (fibrin), minimal hemorrhage and smaller amounts of eosinophilic cellular and karyorrhectic debris (necrosis). Frequently, histiocytes contain dark brown, intracytoplasmic pigment (melanin or hemosiderin). Frequently, throughout the affected tissue, the previously described inflammatory cells are centered upon and efface blood vessels. These are lined by reactive endothelium with frequent obliteration of the vascular wall (vasculitis). Expanding and filling the anterior chamber, posterior chamber and the vitreous is abundant previously described edema, fibrin, fewer inflammatory cells and moderate hemorrhage (hyphema). Diffusely the retina is detached and occasionally the underlying retinal pigment epithelial (RPE) cells are plump and individualized (hypertrophic or tombstoning), with occasional rupture of the RPE and extrusion of pyogranulomatous inflammation into the vitreous. The retina is diffusely disrupted by hemorrhage, fibrin and edema with segmental loss of ganglia, occasional fusion of the inner nuclear and outer nuclear layers, and mild infiltrates of the previously described inflammatory cells (retinitis). Multifocally, both the conjunctiva and orbital muscles



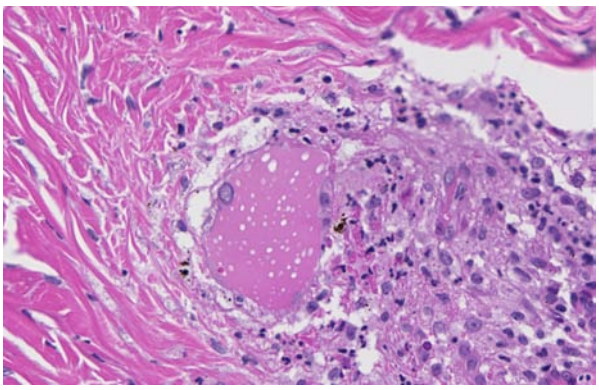
4-2. Eye, cat. A high-protein effusion fills the anterior and posterior segments, detaching the retina (arrows). There is a marked lymphoplasmacytic and histiocytic infiltrate within the uvea (arrowheads). (HE 63X)

have perivascular infiltrates of lymphocytes, plasma cells, macrophages and neutrophils admixed with fibrin and edema. Multifocally, scattered rhabdomyocytes are undergoing degeneration; necrosis, characterized by contraction bands with hypereosinophilic fragmented sarcoplasm lacking cross-striations; or rarely are lost.

Contributor's Morphologic Diagnosis: Eye: Vasculitis, pyogranulomatous and lymphoplasmocytic, multifocal to coalescing, severe with hyphema, panuveitis, scleritis, retinal detachment, mild retinitis, and periocular, perivascular rhabodmyositis and conjunctivitis.

Contributor's Comment: This is a classic example of Feline Infectious Peritonitis Virus (FIP) affecting the eye of a cat. FIP is theorized to originate from mutated enteric coronavirus (FCoV) and can result in a systemic pyogranulomatous vasculitis dependant on the immune status of the affected animal. Feline coronavirus belongs to the family Coronaviridae of the order Nidovirales and, along with canine coronavirus and porcine transmissible gastroenteritis virus, are part of the group I coronaviruses.

While the genetic composition of the coronavirus affects the degree of virulence, the severity of disease is also directly linked to the genetic background of the affected animal. The literature describes a genetic predisposition which suggests that certain subsets of cats are at higher risk for clinical disease than other populations.[2] Evaluation of a large cohort of different cat breeds noted that Birman, Ragdoll, Bengal, Rex, Abyssinian, and Himalayan breeds may be predisposed.[1,8] Similar findings have been noted in



4-3. Eye, cat. Multifocally, areas of histiocytic and lymphoplasmacytic inflammation are centered on vessels. (HE 400X).

cheetah populations which are at increased risk for FIP infection.[3,6,8]

Initial FIP viral replication occurs in the tonsils or intestinal tract, followed by incorporation into histiocytes and transmission to the regional lymph nodes where the virions replicate within macrophages, thereby disseminating the virus throughout the body. The severity of FIPV infection, once the virus is systemic, is determined by the host's immune response. A strong cell-mediated immune response can clear the infection and clinical feline infectious peritonitis will not develop; however, in cats with a poor cell-mediated immune response, antibodies develop which may facilitate macrophage uptake of virus and exacerbate the course of disease. Furthermore, the absence of CMI antigen-antibody complexes results in a type 3 hypersensitivity reaction with associated vasculitis and effusion into body cavities, to include the ocular chambers.[1,2,6]

While a variety of diagnostic modalities have been suggested, there is no gold standard for FIP diagnosis other than histopathology with accompanying immunohistochemistry.[3] However, CBC, blood chemistry, and serology can be highly suggestive of FIP infection and clinically ill cats with FIP may demonstrate the following abnormalities:

- Normocytic, normochromic, non-regenerative anemia
- Neutrophilic leukocytosis with lymphopenia, eosinopenia and monocytosis
- Hyperproteinemia with hyperglobulinemia
- Decreased Albumin:Globulin ratio (increased α2-, β- and γ-globulin concentrations)

Ocular pathology with panuveitis is a common finding with associated iridial changes in iris color, dyscoria or anisocoria secondary to iridocyclitis followed by a sudden loss of vision and clinically observable hyphema. Keratic precipitates colloquially known as 'mutton fat' deposits on the ventral corneal endothelium are reported. On ophthalmoscopic

examination, chorioretinitis, fluffy perivascular cuffing (representing retinal vasculitis), dull perivascular puffy areas (pyogranulomatous chorio-retinitis), linear retinal detachment and fluid blistering under the retina may be observed and these findings mirror those seen microscopically.[1]

As a result of the unique anatomical and functional nature of the eye, pathology within one structure can have significant "bystander" effects on other tissues resulting in progressive destruction and a continuum of pathology from potential inflammation of one region, eventually progressing to complete destruction of the globe. Due to the probable type 3 hypersensitivity associated with the pathogenesis of FIPV in this case, with vasculitis concentrated in the uvea and the sclera, and the resultant effusion into the ocular chambers, the preferential morphological diagnosis defers to the vasculitis as the primary modifier and then identification of each ocular structure in turn affected by the progressive inflammatory process.

JPC Diagnosis: Eye: Panophthalmitis, lymphoplasmacytic and histiocytic, moderate to severe, with uveal vasculitis, anterior and posterior chamber effusions, and retinal detachment.

Conference Comment: Conference participants noted that some areas of the digitized slide were out of focus.

The diffuse uveitis associated with FIP is likely an immune-mediated reaction and, more specifically, a Type III hypersensitivity reaction. Type III hypersensitivity reactions result from the deposition of antigen-antibody complexes in vascular walls, serosa, and glomeruli and result in localized vasculitis from the activation of complement, the recruitment of neutrophils and monocytes, and subsequent damage from the liberation of free radicals and lysozymes. As the leukocyte adhesion cascade becomes activated, macrophages bind to the endothelium and release proinflammatory cytokines, resulting in an acute inflammatory response. Type III hypersensitivity occurs when there is a greater proportion of antigen to antibody, resulting in the formation of medium-sized immune complexes that do not fix complement and are not cleared from the circulation because macrophages are unable to bind them.[7]

Interestingly, FIP-induced ocular lesions are always bilateral. Although FIP typically results in pyogranulomatous inflammation of multiple organs, the inflammatory picture in the eye is unique. The anterior chamber is usually affected by a neutrophilic exudate, while lymphoplasmacytic inflammation is present in the uvea and choroid.[5] Also, in cats, there is no other known cause except FIP for the characteristic inflammation of the extra-ocular muscles combined with uveitis.

In this case, the drainage angle is open, which is to be expected in ocular FIP because the eye is one of the last organs to be infected in this disease, and the cat often dies of severe lesions elsewhere before there is time to develop glaucoma. There is also loss of the inner layers of the retina due to pressure necrosis from the exudate in the vitreous. The detachment of the retina is likely due to effusion in the choroid with leakage into the subretinal space, a process known as exudative retinal detachment.[5] Also present are collagen strands on the anterior surface of the iris, which represent pre-iridal fibrovascular membrane (rubeosis iridis in humans) formation. Pre-iridal fibrovascular membranes are initially composed of polymerized fibrin, hemorrhage, and high protein exudate in the anterior chamber, and are common in acute and chronic ophthalmitis in cats. Pre-iridal membranes originate as endothelial buds from the anterior iridal stroma and mature into fibrovascular membranes that can result in hyphema or glaucoma due to leaky interendothelial junctions of the new vessels resulting in occlusion of the filtration angle or the pupillary opening. This layer of granulation tissue then matures in typical fashion. Pre-iridal fibrovascular membranes are often difficult to identify due to the heavy pigmentation of the iris, and the severe concurrent inflammation that can accompany this phenomenon.[4]

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Case Courtesy of Dr. Lynn Facemire

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