Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 9

18 November, 2020

Joint Pathology Center
Silver Spring, Maryland

CASE 1: A18-11077 (4118172-00)

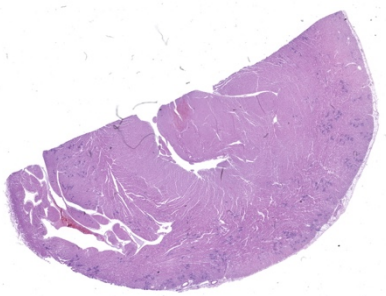
Signalment:
7-yr-old, male (neutered), domestic shorthaired cat (*Felis catus*), cat

History:
An acute episode of hemolytic anemia occurred in the cat five days after a dental prophylaxis. Following a transfusion, the cat improved for several weeks, then became increasingly depressed, anorexic, anemic, and thrombocytopenic, accompanied by vomiting and hematemesis. Euthanasia was elected and a necropsy performed.

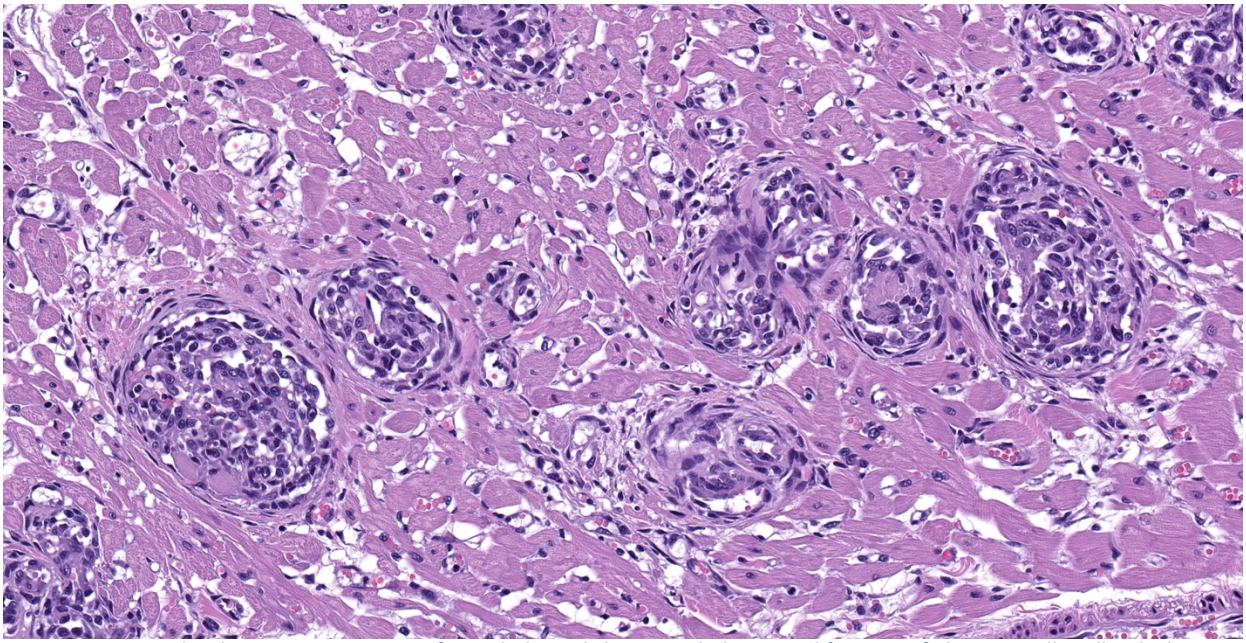
Gross Pathology:
A necropsy was performed on a 7-year-old, 3.5 kg, neutered male, domestic shorthaired cat in fair physical condition. Autolysis was mild. Mucous membranes were pale pink. The heart weighed 18 g (0.51% of body weight), with a 0.6 cm thick left ventricular free wall, 0.1 cm right ventricular free wall, 0.6 cm thick interventricular septum. The lungs were mottled pink to dark red, soft, and air-filled. The liver weighed 100g (2.8% of body weight) and was mottled green-tan to red. The spleen weighed 18 g (0.51% of body weight) and was dark red and plump.

Laboratory results:
N/A

Microscopic description:
Heart: Multifocally, myocardial arterioles in the left and right ventricle and interventricular septum are expanded and occluded by fibrin thrombi, erythrocytes, and intravascular spindle cell proliferation. Spindle cells are often plump, with round to oval nuclei, densely stippled chromatin and pale eosinophilic cytoplasm. The tunica media is occasionally infiltrated by eosinophilic, homogenous material (fibrin) and edema. These arterioles are often surrounded by streams of pale eosinophilic fibrosis and associated fibroblasts, with corresponding myofiber disarray. Approximately 10% of the myocyte population has one of the following changes: degeneration with swollen, hypoeosinophilic, vacuolated sarcoplasm;



Heart, cat. At low magnification, areas of hypercellularity are scattered throughout the outer half of the myocardium in both the left and right ventricle and the interventricular septum. (HE, 5X)



Heart, cat. Arterioles demonstrate a range of morphological change including a proliferation of plump spindle cells which expand and partially occlude the lumen and contain slit-like channels, and occasionally fibrin thrombi. Arteriolar walls are expanded by fibroblasts and collagen and the adventitia is expanded by lamellae of collagen and fibroblasts which occasionally compress or replace adjacent myocardium. (HE, 247X)

necrosis with eosinophilic sarcoplasm, loss of cross striations, and pyknotic nuclei or regeneration with enlarged multinucleation.

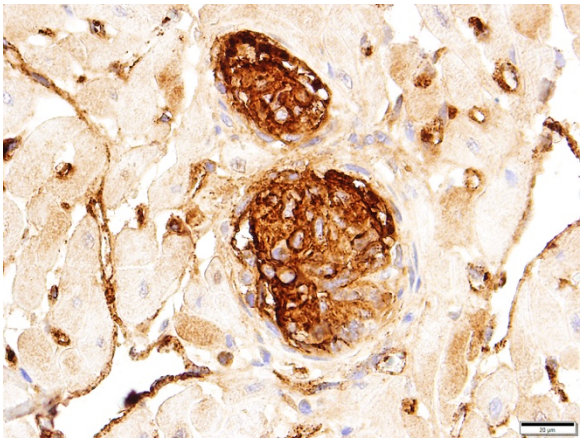
Among the proliferating spindle cell population in the heart, immunohistochemical staining of cytoplasm was widespread and strongly positive for vimentin and von Willebrand's factor. Pericytes and scattered cells within the proliferating cells stained positive for smooth muscle actin. Immunohistochemical staining for *Bartonella* spp. was negative.

Contributor's morphologic diagnosis:
Heart: Intraluminal and mural vascular spindle cell proliferation, multifocal, chronic, moderate to marked; with multifocal thrombosis and perivascular edema
Myocardial degeneration and necrosis, focal, chronic, moderate; with fibrosis

Contributor's comment:
Histologic lesions and immunohistochemistry results are consistent with the feline systemic reactive angioendotheliomatosis (FSRA).

Feline systemic reactive angioendotheliomatosis (FSRA) is a rare, multisystemic intravascular proliferative disorder.[4] Affecting domestic cats almost exclusively, a similar condition has been reported in a Corriente steer.[2,3,4,7,8] Intravascular proliferative disorders in cats are represented by a malignant angiotropic lymphoma, with immunohistochemical properties of T cells, and a condition with histologic and immunohistochemical features similar to human reactive angioendotheliomatosis (RAE). In humans, RAE is a benign process limited to the skin that is characterized by proliferation of intravascular endothelial cells mixed with smaller numbers of pericytes. Similar intravascular proliferations occur in cats as the fatal multisystemic disease, feline systemic reactive angioendotheliomatosis (FSRA).[4] Ultrastructural and immunohistochemical findings reveal the two distinct populations of spindle cells. Lesions are dominated by endothelial cells expressing von Willebrand's factor and smaller numbers of pericytes that express smooth muscle actin.[4,7]

The disease is seen most commonly in juvenile to young adult male cats of no specific breed. Clinical signs and gross lesions are varied and

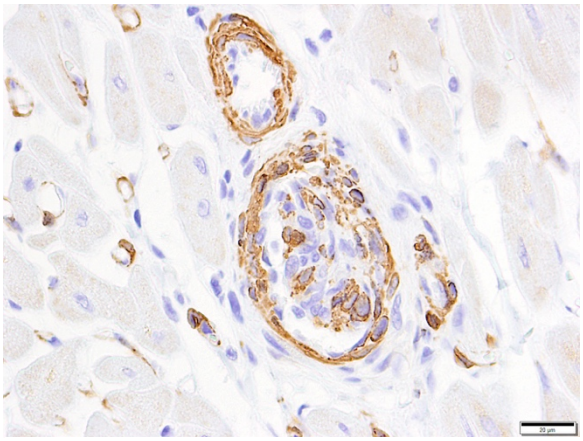


Heart, cat. Spindle cells within arteriolar lumina demonstrate strong positivity for von Willebrand factor (Photo courtesy of: Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 www.vet.uga.edu/VPP) (anti-VWF, 400X)

non-specific, including anorexia, lethargy, weight loss, vomiting, hemorrhages, hematuria, anemia, icterus, seizures and other neurologic signs, thrombocytopenia, and spontaneous death. While a number of these signs, including thrombocytopenia, were present in this case, there was no evidence of respiratory distress, pulmonary edema, pericardial or pleural effusion suggestive of cardiac insufficiency.[3,4,7,8] Affecting primarily small arterioles of the heart, lesions also occur frequently in the kidneys, spleen, lymph nodes, gastrointestinal tract, brain and meninges, eyes, and pancreas. The liver, spinal cord, adrenal glands, thyroid, subcutis, lung, and bone marrow are less commonly affected. Other findings include multifocal fibrin thromboses, hemorrhages, and acute myocardial necrosis. Death is likely the result of cardiac insufficiency.[4] In addition to changes in the heart, fibrin thrombi and/or intravascular spindle cell proliferation was present in arterioles of the brainstem, cerebellum, cerebral cortex, renal cortices, lung, liver, spleen, pancreas, intestinal submucosa, and adrenal glands of this cat.

The pathogenesis of FRSA is poorly understood, although the presence of two cell populations and lack of cellular atypia, suggests a reactive proliferative process rather than a neoplastic one.[4] A number of other occlusive, intra-arteriolar proliferative disorders in humans involve mixed populations of endothelial cells and pericytes. These glomera or glomeruloid structures have involved certain cases of chronic glomerulonephritis, pulmonary hypertension, and cases of chronic disseminated intravascular coagulation and thrombotic thrombocytopenic purpura. Possible mechanisms for the lesion include an exaggerated response to thrombosis, unusual immune-mediated vasculitis, or exuberant angiogenesis related to angiogenic cytokines and/or dysfunctional endothelial regulation of coagulation and fibrinolysis.[4]

Infectious diseases in humans with HIV-AIDS have been associated with proliferative endothelial lesions. These include Kaposi's sarcoma, caused by human herpesvirus-8, and bacillary angiomatosis, caused by *Bartonella henselae* and *B. quintana*. Although information is limited, associations between infection with FIV, FeLV, and FIP virus in cats with FRSA have not been made.[4,7] It has been shown that infection with one or more *Bartonella* sp. may contribute to the pathogenesis of FRSA in cats and hemangiopericytomas in other animals.[1] Immunohistochemical staining of multiple tissues for *Bartonella* spp. was negative in this case.



Heart, cat. Spindle cells within arteriolar lumina multifocally demonstrate strong cytoplasmic positivity for smooth muscle actin. Arteriolar mural smooth muscle serves as a positive internal control. (Photo courtesy of: Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 www.vet.uga.edu/VPP) (anti-SMA, 400X)

Contributing Institution:
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JPC diagnosis:
Heart, arterioles: Atypical endothelial and pericyte proliferation (angioendotheliomatosis), diffuse, severe, with fibrin thrombi and adventitial fibrosis.

JPC comment:
The contributor concisely summarizes this relatively uncommon disease in cats. While the presentation of human reactive angioendotheliomatosis differs from that of cats, they are histologically similar, with both proliferating endothelial cells and pericytes comprising the lesions. In a case report from 2006, a 57-year-old intact woman was diagnosed with cutaneous reactive angioendotheliomatosis. Her medical history included a history of vascular disorders which included both deep vein thrombosis and a significantly lower measured blood pressure on her left arm, with a bruit of the left subclavian artery. She also had elevated anticardiolipin antibody IgG and decreased protein C levels. Activated protein C is a critical factor in anti-coagulative effects, and anticardiolipin antibodies has been shown to lower levels of protein C, leading to pro-coagulative states. In humans, anticardiolipin antibodies are found in approximately 23% of patients with Systemic Lupus Erythematosus (SLE) and is associated with thrombotic events. It has been suggested that one possible pathogenesis of this disease is vascular occlusion leading to localized hypoxia and acidosis, followed by hyperplasia of endothelial cells and pericytes in an attempt to recanalize vessels and restore perfusion of tissues.[5]

While too few cases of FSRA have been reported to make subclassifications, there have been proposals to make distinctions in possible subtypes of human reactive angio-endotheliomatosis. Subtypes may include diffuse dermal angiomatosis (DDA), acroangiodermatitis, glomeruloid angio-endotheliomatosis, and angiomatosis associated with cryoproteins (also called angiopericytomatosis). A more recent proposal is to include an entity characterized by a benign proliferation of histiocytes within the lumena of cutaneous vessels (intravascular histiocytosis).[6]

While it cannot be stated definitively that human reactive angioendotheliomatosis and FSRA share a similar pathogenesis, there is biologic plausibility. Perhaps these young cats experience an endotheliotropic pathogen, or a subsequent immune complex disease, which alters Virchow's triad and their anticoagulative homeostasis. A potential direction to turn for research may be immunohistochemistry for HIF-1 and VEGF, as well as the incorporation of clinicopathologic data for recorded cases.

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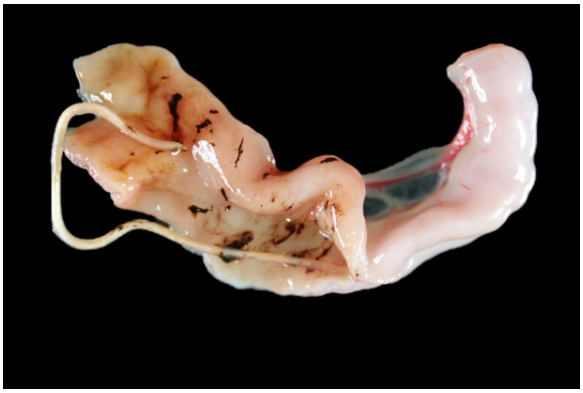
CASE 2: Case 2 (4153150-00)

Signalment:
8-week-old, male entire, domestic short hair cat (*Felis catus*)

History:
This kitten was found on the side of the road and was presented to the Massey University Pet Emergency Centre by a member of the public. On arrival to the clinic, the kitten was hypothermic and hypoglycemic. Following administration of IV fluids and dextrose, the kitten recovered and was sent to the SPCA.

A week later, the kitten re-presented to the clinic in lateral recumbency with bradycardia, evidence of severe diarrhea (perineal staining) and agonal gasping. Due to poor prognosis, euthanasia was elected.

Gross Pathology:
The kitten was in an emaciated state of nutrition (with a body condition score of 1/9 and a total body weight of 0.514 kg) and was markedly dehydrated with markedly prolonged skin tenting and marked enophthalmos. Significant fecal staining around the perineum, under the tail and on the hindlimbs was present.



Intestine, cat. The intestine displays diffuse pallor, luminal digested blood, and a large luminal ascarid. There is minimal mesenteric fat. (Photo courtesy of: Massey University, School of Veterinary Science, New Zealand)

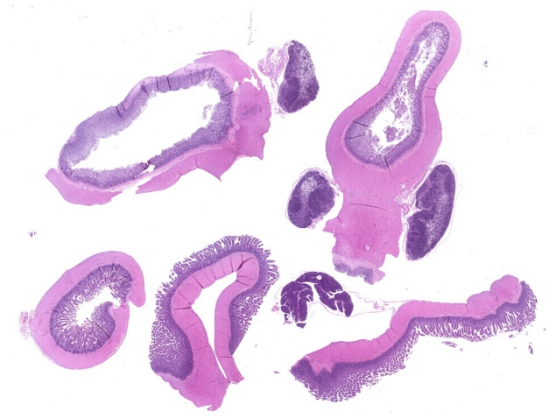
On internal examination, the stomach was empty except for red-brown mucus over the pyloric mucosa. The intestines were mostly empty except for scant amounts of reddish-black granular material (digested blood) loosely adhered to the intestinal mucosa. There were also 5 white nematode parasites ranging between 3 and 5 cm long in the small intestine (*T. cati*). The colon contained scant, unformed reddish-brown feces. No other gross lesions present.

Laboratory results:
None.

Microscopic description:
Small intestine: Villi and crypts are segmentally effaced and replaced by extensive infiltrates of neutrophils, large colonies of small uniform Gram-negative bacilli, fibrin, hemorrhage and cellular debris. Remaining crypts within these are areas are ectatic, lined by cuboidal to attenuated epithelial cells, and contain neutrophils, sloughed enterocytes and cellular debris within luminal spaces. Other crypts contain hyperplastic, piling epithelium with increased numbers of mitotic figures (regeneration).

Enterocytes within the crypts multifocally have marginated nuclear chromatin and bright eosinophilic intra-nuclear inclusions (viral inclusions). Adjacent mucosal villi are mildly to moderately blunted and fused with increased numbers of neutrophils and macrophages expanding the lamina propria. A focal area of the submucosa is infiltrated by large numbers of degenerate neutrophils and Gram-negative bacilli. Peyer's patches show marked lymphoid depletion with the presence of lymphocytolysis and large numbers of tingible body macrophages within follicular centers.

In one section of jejunal lumen is a 1 mm x 2mm cross-section of a nematode larvae with coelomyarian musculature, a ridged cuticle, intestinal tract, uteri containing approximately 15 -20 eggs each and ovarian structures.



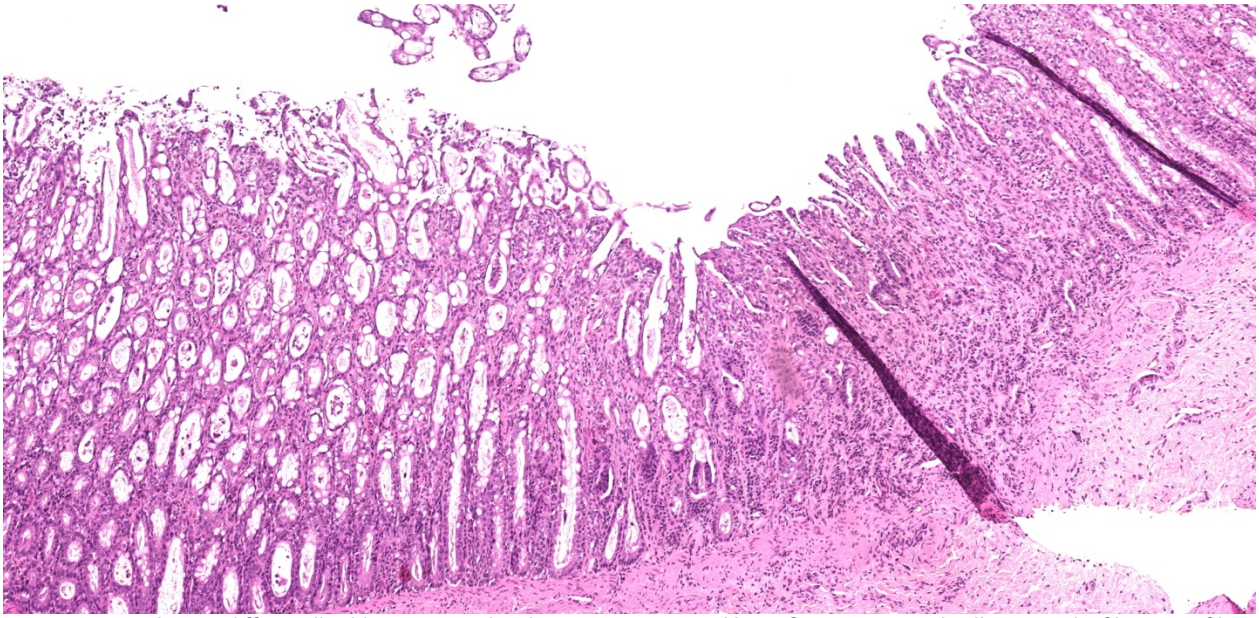
Multiple tissues, cat. Multiple sections of jejunum, lymph nodes and a section of pancreas are submitted. Even at low magnification, several sections of intestine display marked villar blunting. (HE, 7X)

Contributor's morphologic diagnosis:
Small intestine: Severe, acute segmental necrosuppurative enteritis with crypt necrosis, intra-nuclear viral inclusions, Gram-negative bacilli and nematode larvae

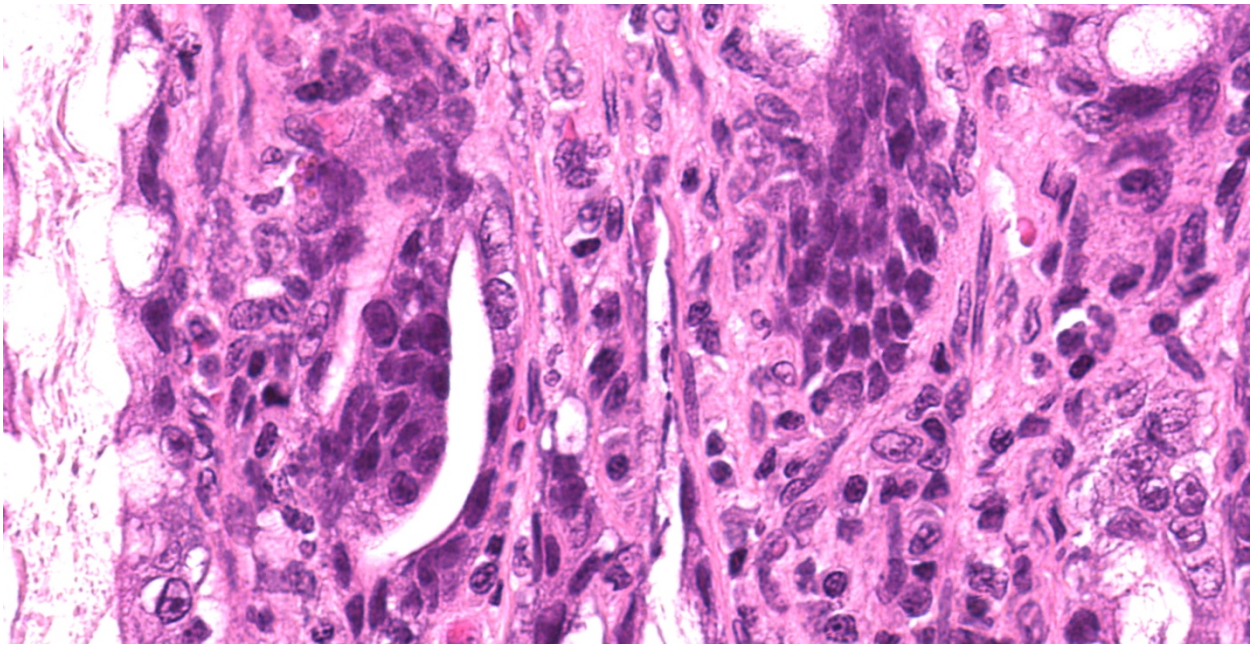
Contributor's comment:
The intestinal lesions in this case are strongly suggestive of feline panleukopenia and secondary bacterial enteritis which were likely significant contributors to the poor body condition and diarrhea in this kitten. Small numbers of *Toxocara cati* were also present in the small intestine, which may have further contributed to the emaciation and diarrhea but were not causing obstruction of the bowel and were unlikely to be the leading cause of death.

Feline panleukopenia virus (FPV) is a member of the feline parvovirus subgroup within the Parvovirus genus (family Parvoviridae) and is closely related to mink enteritis virus (MEV) and canine parvovirus-2 (CPV-2).[3] FPV is a relatively small (25nm) non-enveloped single-stranded DNA virus with a genome of approximately 5000 bases.[12] Due to the highly stable nature of FPV within the environment, the large quantities of virions shed in feces (10^7-10^9 per gram), and the ability of the virus to remain infectious for weeks post-shedding, FPV is considered an ubiquitous pathogen found in almost all regions of the world.[8]

Although FPV has been proven to affect cats of all ages, kittens tend to be most susceptible to the disease.[11] The predisposition of kittens contracting the disease is due to kittens experiencing a precarious period of sub-optimal immunity against the virus between 8-12 weeks of age where levels of circulating maternally



Jejunum, cat. There is diffuse villar blunting at right, there is transmucosal loss of crypts, stromal collapse, and infiltration of low numbers of neutrophils and macrophages. At left, there is multifocal dilation of crypts by necrotic epithelium and debris. (HE, 77X)



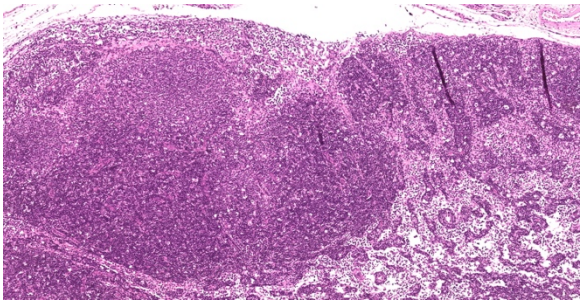
Jejunum, cat. Nuclei of crypt epithelium in areas of crypt necrosis are karyomegalic and hyperchromatic, suggesting the possibility of viral intranuclear inclusions. (HE, 400X)

derived antibodies (MDA) are insufficient to protect against infection yet high enough to interfere with vaccination.[11] Despite most cats not developing clinical disease following infection, those who do develop overt disease are at great risk of death with mortality rates of ≥ 90% reported in the literature.[11]

The most common clinical signs observed in affected cats include anorexia, diarrhea, vomiting, lethargy and pyrexia.[7] Co-infection of the host with intestinal parasites (such as *T. cati* in this case), bacteria, or other viruses (e.g. rotaviruses or coronaviruses) are likely to increase the severity of the disease.[8] Infected cats tend to die from complications associated with translocation of bacteria into the bloodstream, sepsis and disseminated intravascular coagulopathy (DIC).[5] The most common laboratory abnormalities present in affected cats include leukopenia, thrombocytopenia, anemia, neutropenia, hypoalbuminemia and lymphopenia.[7] It is important to note that kittens infected late during their development in utero exhibit a distinct manifestation of the disease characterized by nervous signs (namely ataxia and hypermetric gait) due to viral induced cerebellar hypoplasia.[13]

The route of entry of FPV into the host is oronasal with subsequent uptake of the virus into the epithelium overlying the tonsils and/or Peyer's patches.[13] Following host inoculation, lymphogenous spread of the virus may result in infection of other lymphatic structures including the thymus, spleen and lymph nodes. Virally induced lymphocytolysis within these lymphatic structures induces cell-free hematogenous spread of the virus to a variety of different organs throughout the body. It is late during this viremic period (approximately 5-7 days post infection) in which most clinical signs are observed.[13]

As FPV requires the 'hijacking' of host cell DNA polymerases and other DNA replication machinery, the virus has a tropism for mitotically active cells within the host.[12] The predominant cells infected by FPV outside of lymphoid tissue are enterocytes in crypts of Lieberkühn adjacent to or near Peyer's patches and stem cells within the bone marrow.[12] Invasion of FPV into these mitotically active host cells is achieved through endocytosis mediated by viral capsid proteins binding to host neuraminic acid and transferrin receptors on the cell surface.[13] Once within the host cell, the virus is only able to replicate during the S-phase of the DNA replication cycle. Following viral replication, FPV virions are



Mesenteric lymph node, cat. The adjacent lymph node, especially in light of profound intestinal necrosis in the adjacent organ, is hypocellular, lacks follicle formation, and contains numerous tingible body macrophages. (HE, 80X)

released from infected cells with subsequent lysis and death of the host cell.

In the case of infected enterocytes, lysis of these cells results in the release of virions into the intestinal lumen thus contaminating the feces. Destruction of enterocytes results in a markedly reduced absorptive surface within the small intestine and subsequent diarrhea. It should also be noted that effusion of tissue fluids and blood from denuded intestinal mucosa into the lumen is also hypothesized as a contributing cause of diarrhea in FPV infected patients.[13] Anemia and hypoproteinemia are also hypothesized to result from effusion of tissue fluids and blood from denuded intestinal mucosa into the lumen. The hallmark panleukopenia observed in individuals infected by FPV is the result of both lymphocytolysis within lymphoid tissues and destruction of myeloid precursors in bone marrow. Histopathologically, lysis of stem cells within the bone marrow is appreciated as marked inter-trabecular hypocellularity.[13]

Additional confirmatory diagnostic tests for FPV include immunohistochemistry of affected tissue, fecal ELISA, fecal PCR, fecal latex agglutination test (LAT) or fecal immunochromatography.[11,13]

Contributing Institution:
Massey University
School of Veterinary Science
New Zealand

JPC diagnosis:

1. Small intestine: Enteritis, necrotizing, diffuse, severe, with villar blunting, crypt hyperplasia, crypt abscesses, lymphoid depletion, and rare intranuclear viral inclusions.
2. Lymph node: Lymphoid depletion, diffuse marked.

JPC comment:
A brief discussion was held regarding positively charged glass slides, and how important these are for tissue retention during processing. Positively charged slides are marked with a "+" and are purchased with this feature. A number of tissues have been lost during processing of immunohistochemical stains, and this feature greatly increases the likelihood of not losing the tissue.

The contributor provides a broad overview of feline panleukopenia and many of the presentations of this disease. Feline panleukopenia is the oldest known viral disease of cats and is a well-known pathogen around the world. *Carnivore protoparvovirus 1* is the base virus previously described, which contains two open reading frames (ORFs). ORF-L codes for two non-structural proteins, NS1 and NS2; ORF-R codes for two structural proteins, VP-1 and VP-2 (major capsid protein). Feline parvovirus, canine parvovirus, mink enteritis virus, and others are different strains of *carnivore protoparvovirus 1*, but there is a high degree of homology between the sequences of their capsid proteins.[1,2] Binding of the transferrin receptor (TfR) is crucial for productive infection, which then allows for the virus to be taken into the host cell through clathrin-mediated endocytosis.[1]

While closely related, canine parvovirus 2 (CPV-2) differs from feline panleukopenia virus by 6 amino acids in the VP-2 region, preventing efficient binding of canine TfR and clinical disease does not develop. However, there are a number of variants of CPV-2 that are capable of binding both canine and feline TfRs and leading to disease in both species (CPV-2a, CPV-2b, CPV-2c).[1]

In recent research, an additional histologic finding has been cytoplasmic vacuolation of

neurons in the thoracic spinal cord of an infected cat. Using advanced sequencing techniques, feline panleukopenia virus antigen was recovered from vacuoles, as well as neuronal cytoplasm, suggesting that neuronal vacuolation is a histologic change consistent with feline panleukopenia virus infection.[9]

There have been confirmed cases of feline panleukopenia virus causing disease in other species. There is a high degree of homology between mink enteritis viruses (MEV-1, MEV-2, MEV-3) and feline panleukopenia virus. A recent phylogenetic analysis of viruses isolated from a mink farm suggested that several strains of MEV would more appropriately be considered FPV, instead.[4] More recently in Thailand, a banded linsang presented with signs consistent with parvoviral enteritis, and tested PCR positive for CPV. However, upon sequencing the VP2 gene of the virus, it was determined to be FPV, similar to strains reported in Japan and South Korea.[6] These overlapping test results highlight the similarity of these viruses.

In another disease, a parvovirus has been recently implicated as the most plausible etiologic agent. Theiler's disease in horses, also known as equine serum hepatitis, is characterized by an acute, massive, diffuse hepatic necrosis that is often life-threatening. A number of agents have been implicated, and it appeared to have a correlation with equine serum derived biologics, including tetanus antitoxin, botulinum antitoxin, *Streptococcus equi* antiserum, pregnant mare's serum, and equine plasma. Two flaviviruses were suggested as the cause and included non-primate hepacivirus (NPHC) and Theiler's disease-associated virus (TDAV). However, additional analysis has shown that a startling number of biologics have been PCR positive for Equine parvovirus-hepatitis (EqPV-H). While there were other positives for NPHV, TDAV, and Equine pegivirus (EPgV), all samples of affected horses were PCR positive for EqPV-H.[10]

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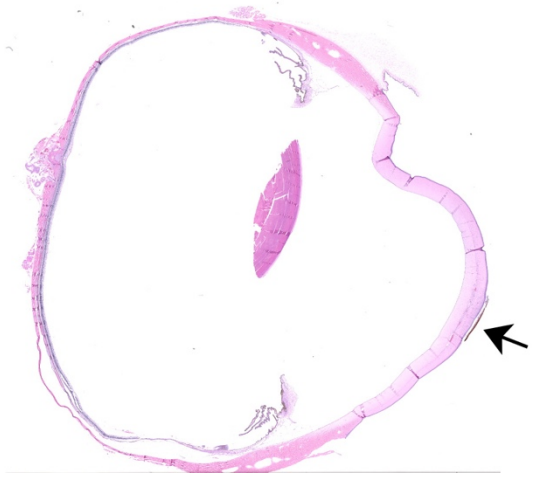

Globe, cat. At subgross magnification, a focal dark brown plaque (arrow) covers the cornea. (HE, 5X)

CASE 3: P17-1884 (4117377-00)

Signalment:
1-year-old male neutered DSH cat (*Felis catus*)

History:
A black spot was noted on the eye by the owner and the eye was enucleated.

Gross Pathology:
The cornea has a 0.5 mm diameter black spot on the surface.

Laboratory results:
None.

Microscopic description:
Adherent to the ulcerated cornea is a plaque of acellular brown-staining, fibrillary material. The corneal ulcer has a bed of granulation tissue extending half the thickness of the stroma. Neutrophils cover the ulcerated surface and extend through the granulation tissue. A few neutrophils are in the surface plaque. The corneal epithelium at the margin of the ulcer has hyperplasia.

Contributor's morphologic diagnosis:
Corneal sequestrum
Ulcerative, suppurative keratitis

Contributor's comment:
A corneal sequestrum is an aggregate of degenerate corneal stromal collagen which becomes pigmented with amber to black pigment. It has also been called partial corneal mummification or corneal nigrum.[3,5-7]

The cause of corneal sequestrum is not known. It may be a response to corneal irritation or be associated with ocular trauma.[3,5-7] The source of the pigment also is not known. One investigator found melanin in the sequestrum[2], but others have not substantiated this.

Corneal sequestrum occurs more commonly in brachycephalic breeds, Siamese, and domestic short hair cats.[5-7] The condition is usually unilateral but can be bilateral in the predisposed breeds.[3]

The ulcerative keratitis is a response to the collagen degeneration of the sequestrum. The sequestrum develops in the stroma but is gradually extruded through the epithelium and comes to lie on the surface of the cornea.

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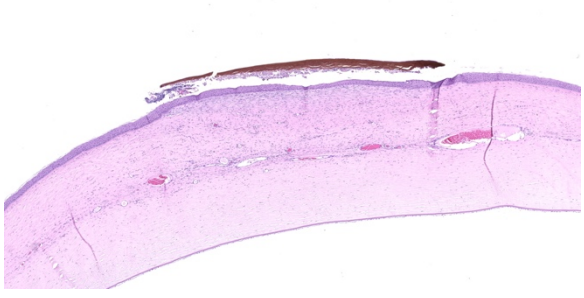
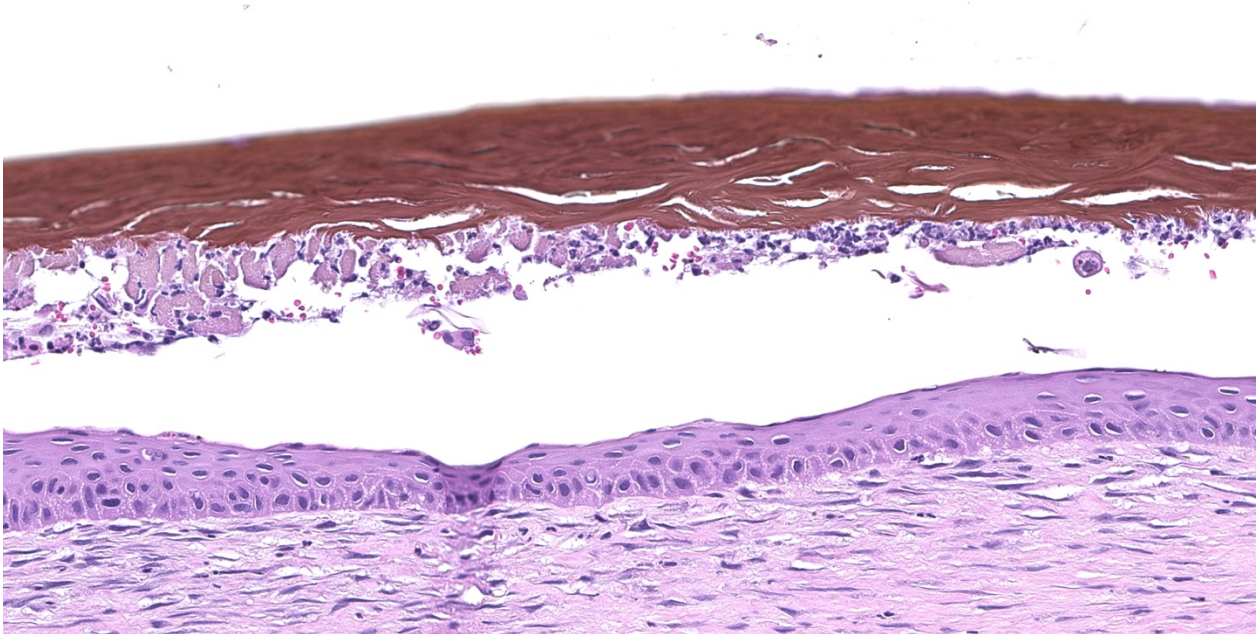
JPC diagnosis:
Cornea: Necrosis, coagulative, focally extensive, with pigmentation and vascularization.



Cornea, cat. Higher magnification of the full thickness of the cornea. At top, a dark brown plaque of necrotic cornea (corneal sequestrum) overlies intact epithelium. There is hyperplasia of stromal cell nuclei, and vascular ingrowth within the middle layers of the cornea. (HE, 31X)



Cornea, cat. Higher magnification of corneal sequestrum. The outer layers are anucleate and dyed a deep brown due to cellular imbibition of porphyrin pigment. The deeper layers of epithelium are swollen and infiltrated by low numbers of neutrophils. The underlying epithelium is intact and there is hypertrophy of stromal cell (keratocyte) nuclei, mild edema, and few infiltrating neutrophils. (HE, 275X)

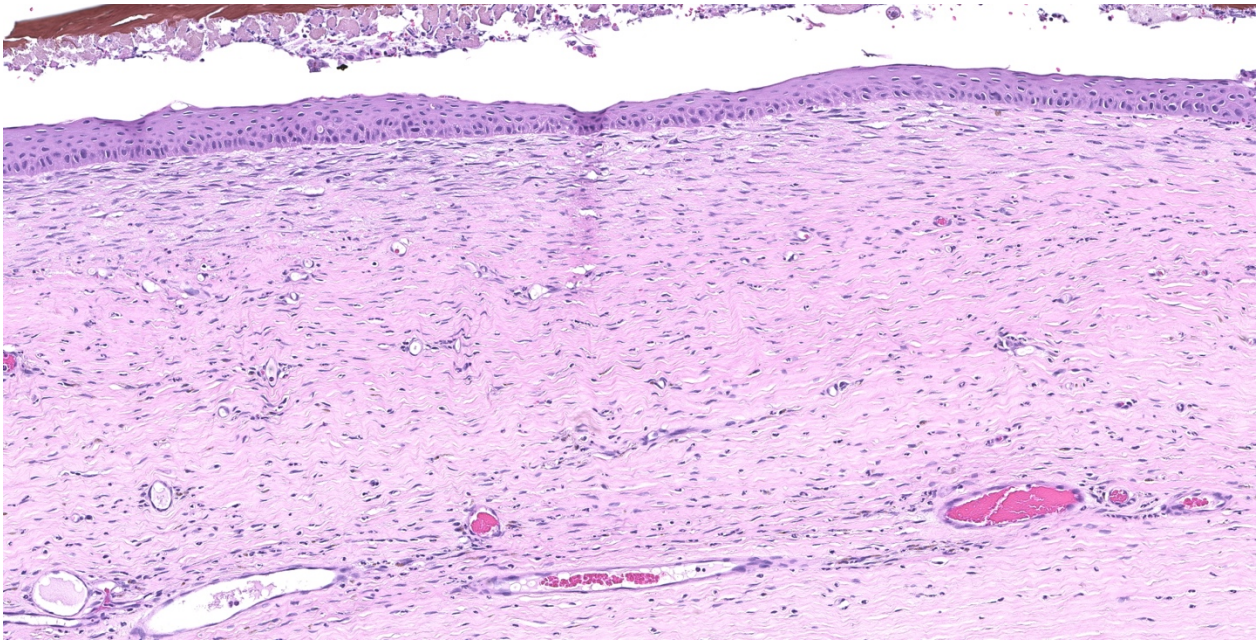
JPC comment:
There is some slide variation between participants, with some sections containing colonies of cocci adherent to the sequestrum. The moderator also briefly discussed picrosirius red, a histochemical stain that allows for the differentiation between healthy and degenerative collagen fibers. Unfortunately, that histochemical stain was not available for this case.

The pathogenesis of feline corneal sequestrum is incompletely understood. However, as the contributor stated, brachycephalic breeds, specifically Persian and Himalayan cats, are more often affected. While early cases may lack the typical discoloration, lesions are characterized by a discrete focus of non-inflammatory necrosis of corneal stromal keratocytes. The stroma affected usually exhibits pallor, hyalinization, and orange discoloration. Sequestra may be accompanied by a concurrent ulceration of the overlying corneal epithelium, or there may be evidence of prior ulceration.[9]

Suggested predisposing factors leading to corneal sequestra include corneal trauma, topical corticosteroid use, primary corneal dystrophy, altered corneal stromal metabolism, chronic corneal ulcers or keratitis, often by feline herpesvirus-1 (FHV-1) or mechanical irritation from entropion or trichiasis.[1,8] During routine histologic analysis, apoptotic keratocytes have been noted, possibly suggesting a role for apoptosis in the development of these lesions.[8]

On transmission electron microscopy (TEM), sequestra contained variable amounts of amorphous, electron-dense substance, continuous with intact basement membrane to the periphery. Remnants of necrotic keratocytes were observed between loosely packed collagen bundles subjacent to ulceration. Occasionally, keratocytes were characterized by clumped and marginated chromatin, with shrunken cytoplasm, changes consistent with apoptosis.[1]

Studies comparing the association of FHV-1 with corneal sequestrum in Persian and Himalayan cats did not find a correlation, with the control group having much higher rates of PCR positive results for FHV-1.[8] Additionally, there does not appear to be an association between qualitative tear film abnormalities, abnormal goblet cell



Cornea, cat. Higher magnification of the outer half of the cornea underlying the sequestrum. The epithelium is intact, and there is mild edema, hypertrophy of keratocyte nuclei, and vascularization of the middle layers of corneal stroma, with infiltration of neutrophils. (HE, 133X)

numbers, *Chlamydia psittaci*, or *Mycoplasma felis*.[4]

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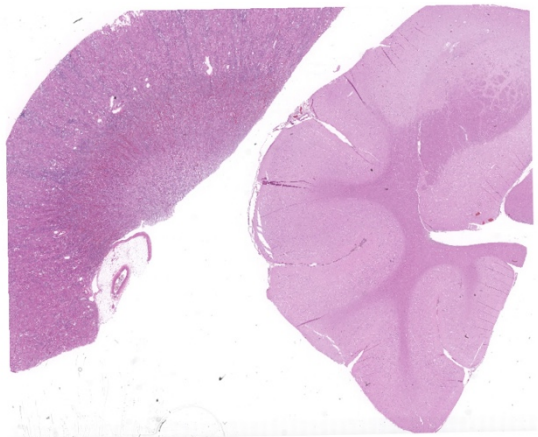
CASE 4: N1929202 (4153152-00)

Signalment:
Adult, intact male, domestic shorthaired cat, *Felis catus*, Feline.

History:
Three cats (two intact males and one intact female) were found dead in the same area and were suspected to have been poisoned. The cats were submitted for postmortem examination.

Gross Pathology:
All cats were in good body condition with adequate fat stores. Granular, dark red-black blood was detected in the stomach of each cat. One cat had numerous pinpoint bleeding gastric



Kidney, cerebrum, cat: Sections of kidney and cerebrum are submitted for examination (HE, 5X)

erosions/ulcers. A different cat had multifocal erosions in the urinary bladder erosion and the lumen contained a coagulum of fibrin and necrotic debris. The kidneys of all three cats were grossly unremarkable.

Laboratory results:
Gas Chromatography Mass Spectrometry (GC-MS) was performed on fresh kidney tissue and ethylene glycol was detected above a level of 50 ppm.

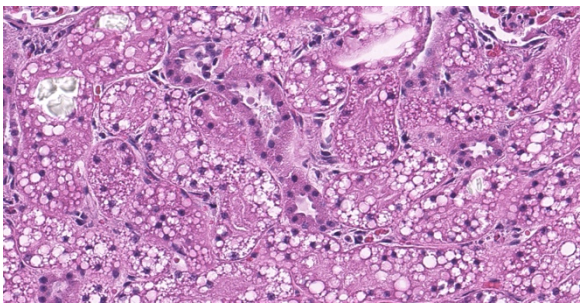
Microscopic description:
Multifocally, numerous renal cortical, and to a lesser extent medullary, tubules are distorted and expanded by radiating colorless crystalline material that is birefringent under polarized light (calcium oxalate crystals). The crystals often fill tubular lumina and disrupt tubular epithelia. Affected tubular epithelial cells are attenuated and degenerate or necrotic with shrunken with hypereosinophilic cytoplasm and pyknotic nuclei. Necrotic cells are occasionally sloughed into the lumen, admixed with cellular debris and eosinophilic fluid. There are few granular and cellular casts and renal epithelia are swollen and contain variably discrete lipid vacuoles (renal lipidosis).

Within the cerebral parenchyma and leptomeninges, birefringent calcium-oxalate crystals multifocally expand the perivascular space, disrupt vessel walls, or rarely, protrude into the vascular lumen. Disrupted vessel walls are replaced by crystals, karyorrhectic debris, and neutrophils. Neutrophils extend into Virchow-Robins space along with small eosinophilic droplets (edema). In less severely affected blood vessels, endothelia are hypertrophied. There is a mild generalized increase in glial cells, predominately astrocytes.

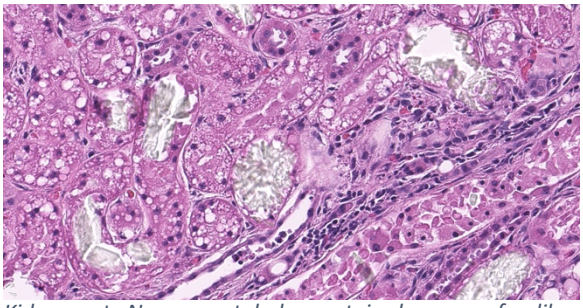
Contributor's morphologic diagnosis:
Kidney, cat: severe acute nephropathy characterized by numerous intratubular crystals, multifocal acute tubular degeneration and necrosis

Brain, cat: numerous perivascular crystals with perivascular and leptomeningeal neutrophilic and lymphohistiocytic infiltrates

Contributor's comment:
Voluntary ingestion of sweet-tasting antifreeze by cats and dogs is the most common presentation of ethylene glycol (EG) toxicosis. Temperate and cold climates during the Fall and Spring have the highest incidence of cases, coinciding with the highest use of this product.[2] Ethylene glycol and its metabolites result in three overlapping stages of disease: central nervous system depression and gastrointestinal upset, metabolic acidosis, and finally acute tubular injury and renal failure.[14] EG itself is relatively non-toxic and systemic effects are due to ethylene glycol metabolites.[7,8] Death can occur predominantly from development of severe metabolic acidosis and acute renal failure. Hyperkalemia from renal failure can induce cardiac arrhythmias and metabolic acidosis can lead to central nervous system depression/seizures/coma and pulmonary edema causing respiratory distress and dysfunction.[14]



Kidney, cat. Diffusely, tubular epithelium is expanded by numerous clear vacuoles. An aggregate of calcium oxalate crystals is present within the lumina of a tubule at upper left (arrow). (HE, 396X)



Kidney, cat. Numerous tubules contain sheaves or fan-like aggregates of birefringent crystals within their lumina. (HE, 400x)

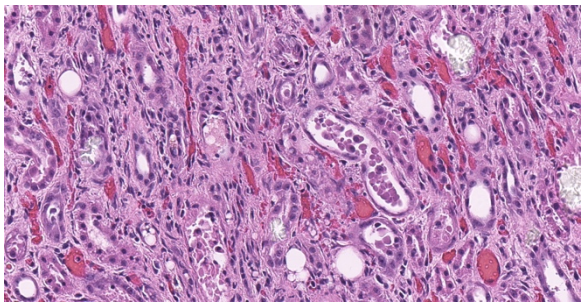
Lethargy, anorexia, dehydration, vomiting, diarrhea, uremic ulcers, and salivation are other common symptoms that can occur.[14]

The metabolism of EG involves a series of rate-limiting and non-rate-limiting steps in the liver and kidneys. Absorption through the digestive tract is rapid, and the majority of EG is excreted through the kidney without being metabolized. The remainder of EG is converted to glycolaldehyde by alcohol dehydrogenase (rate-limiting step), quickly oxidized to glycolic acid, and then oxidized again to glyoxylic acid (rate-limiting step).[8,12,14] Metabolism ends with the production of several compounds, of which oxalate and lactic acid are the most clinically important. Oxalate's importance is discussed in the following paragraph. Lactic acid contributes to the development of metabolic acidosis. The other end metabolites are hippuric acid and carbon dioxide.[14] Once EG has been completely metabolized, treatments aimed at inhibiting alcohol dehydrogenase will not be effective. Prognosis at this stage of disease is guarded to poor.[14]

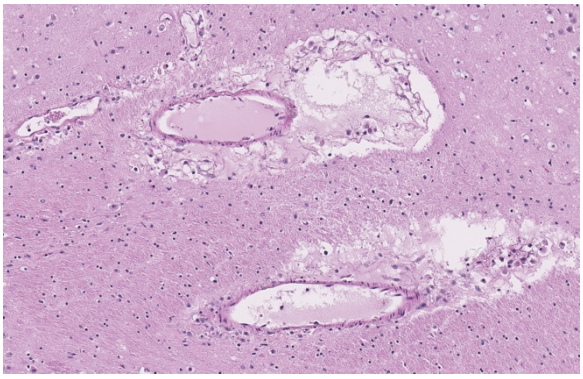
Calcium binds to oxalate and forms crystals within renal tubular lumina and is taken up by tubular epithelium and secreted into the renal interstitium.[1,2] With decreased renal function, crystals will accumulate at high concentrations in circulation and deposit in perivascular spaces within other tissues such as the brain. Glycolaldehyde and glyoxylic acid are classically postulated to be primary effectors, though there has been some debate about the role of EG metabolites in causing direct cellular injury.[7,12] Within the past two decades, multiple studies suggest that deposition of calcium oxalate crystals may be a larger contributor to acute tubular injury and development of renal failure through various proposed direct and indirect cytotoxic mechanisms.[7,10,11]

Clinicopathologic findings include a titrational metabolic acidosis due to the production of acidic metabolites and lactic acid (resulting in a markedly increased anion gap) and severe serum hyperosmolality with an increased osmolal gap.[14] The urine is isosthenuric, acidic, and contains variable numbers of calcium oxalate monohydrate crystals beginning 3-6 hours after ingestion of the toxin.[9,13] Few numbers of monohydrate crystals are normally found in the urine, but high numbers should increase the suspicion of EG toxicosis.[9] Additional biochemical findings are transient hyperglycemia, hypocalcemia, and hyperphosphatemia, which is initially caused by phosphate rust inhibitors in the antifreeze and then persists due to renal dysfunction.[9,14]

At postmortem examination, swollen edematous kidneys, pulmonary edema, and hemorrhagic gastroenteritis are common gross findings, although in many cases gross lesions are not observed.[1,2] Histologically, numerous birefringent calcium oxalate crystals within the renal tubules are the most characteristic and almost pathognomonic finding in cases of EG toxicosis. Primary hyperoxaluria, a rare inherited condition in humans, cats, dogs (Tibetan spaniels, Shih Tzu), and Beefmaster cattle, or ingestion of oxalate containing plants or oxalate producing fungi (such as *Aspergillus* spp.) are other differentials in these cases.[2]



Kidney, cat. A range of tubular epithelial changes, from necrosis to granular cast formation is present within crystal-laden tubules. (HE, 400X)



Cerebrum, cat. Virchow-Robin's spaces within the cerebrum are markedly expanded by edema. (HE, 200X)

An interesting histologic finding in these cats was the detection of perivascular crystals in the brain. The literature postulates that development of central nervous system signs is most likely because of aldehyde and the development of metabolic acidosis rather than the presence of the crystals.[2] Few human case reports highlight delayed neurological effects from cerebral edema and obvious histologic vascular injury suggested to be due to the presence of crystals.[5,6] At this point in time to the author's knowledge, primary research specifically examining effects of crystal formation on vessels and development of neurological signs is limited. In these cats, neutrophilic infiltrates were sometimes observed in association with perivascular crystal deposition. Inflammation was not present in all cats and was variable between cerebral sections. Immunohistochemical staining for feline infectious peritonitis (FIP) was negative. In this case, the inflammatory reaction was suspected to be secondary to vascular damage due to crystal deposition rather than a separate primary process.

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JPC diagnosis:

1. Kidney, tubules: Degeneration and necrosis, diffuse, marked, with numerous intratubular oxalate crystals, and granular and protein casts.
2. Cerebrum, perivascular areas: Edema, multifocal, moderate with neutrophilic and lymphohistiocytic infiltrates, and oxalate crystals.

JPC comment:
The contributor provides an excellent review of this condition in animals. Recent research has investigated the use of stiripentol, an antiepileptic drug used to treat Dravet syndrome in humans, to reduce the formation of calcium oxalate crystals upon ethylene glycol ingestion. Stiripentol inhibits lactate dehydrogenase 5, the final step of hepatic oxalate formation. Sixteen Sprague Dawley rats received hydroxyproline and calcium in water for 16 days, with half receiving stiripentol daily. The experimental group had lower resultant urine oxalate, suggesting a potential future therapy.[3]

Interestingly, while ethylene glycol leads to the disease described in this case, (poly)ethylene glycol is a polymeric molecule with properties that warrant investigation as medical therapies. *In vitro* exposure of metastatic melanoma cells (human, A375 cells) to different molecular weight (poly)ethylene glycol polymers resulted in morphologic signs of toxicity. This is far from a clinical intervention to date but may lead to interesting therapies in the future.[13]

While in veterinary medicine, we typically think of ethylene glycol in the context of automobile antifreeze. But ethylene glycol is also integral to the production of numerous items, such as polyethylene terephthalate (PET) plastics, deicing fluid for aircraft operations, and as an inhibitor of clathrate hydrate formation in natural gas pipelines. With such diverse applications, many involving open-air release, ethylene glycol is a common environmental pollutant that is slow to chemically decompose. Bacteria have been leveraged to assist with the breakdown of numerous plastics, and an engineered strain of *Pseudomonas putita* has shown promise for degrading ethylene glycol to non-harmful byproducts. Specifically, *P. putita* KT2440 was engineered to overexpress glycoxylate carboligase (*gcl*) in combination with products of four other genes in close proximity (*hyi*, *glxR*, *ttuD*, *pykF*), resulting in efficient growth and

utilization of ethylene glycol substrate. Research in this vein may assist in removing this toxic compound from the environment in the future.[4]

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