Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2014-2015

Conference 3

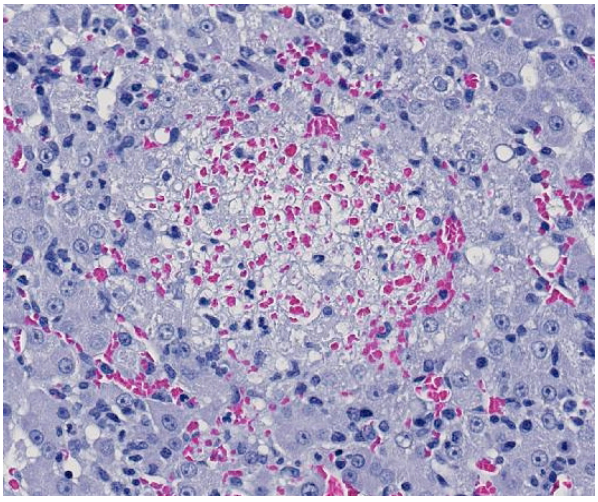
17 September 2014

CASE I: 12-3003 (JPC 4019855)

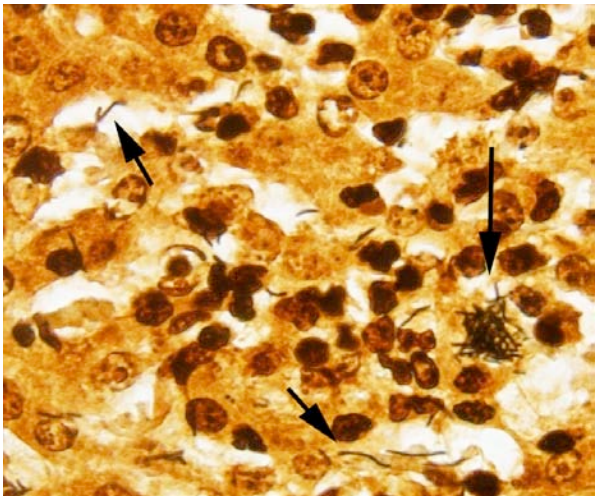
Signalment: 8-week-old female domestic shorthair feline (*Felis catus).*

History: The kitten reportedly developed pale, unformed stools, anorexia, rapid weight loss and death within 2 weeks of purchase from a pet store. The owner of the pet store reported that multiple kittens both living at the store and recently purchased from the store had developed similar clinical signs, with most of the affected kittens recovering after supportive treatment. In addition, two apparently well-grown kittens had died suddenly within the previous 2 months.

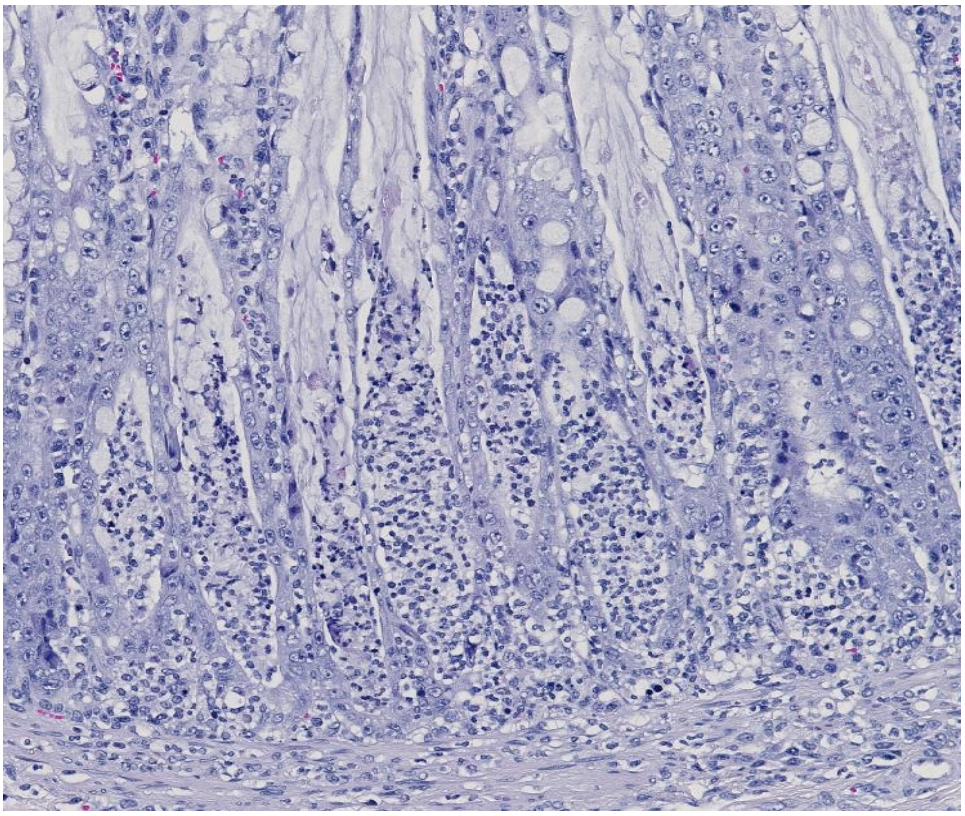
Gross Pathology: The kitten presented within 12 hours of death and was judged to be in an adequate state of post-mortem preservation, in very poor body condition (315g) and moderately dehydrated. Pasty, yellow-grey feces stained the tail and perineum. A small amount of kibble was



1-1. Liver, kitten: Multiple foci of necrosis are infiltrated by low numbers of neutrophils. (HE 164X)



1-2. Liver, kitten: Filamentous bacilli consistent with Clostridium piliforme are present within hepatocytes and extracellularly within necrotic foci. (Warthin-Starry 4.0, 60X)



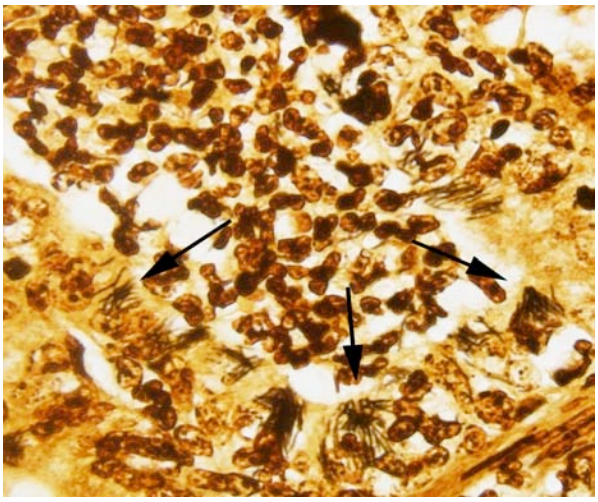
1-3. Colon, cat: Colonic glands are diffusely expanded and contain numerous necrotic epithelial cells, degenerate neutrophils, and moderate amounts of mucus (crypt abscesses). (HE 120X)

present in the stomach. The intestine and colon contained scant yellow mucoid content. There was patchy reddening of the serosal surface of the small intestine and proximal colon. Moderate numbers of pale, randomly scattered 1-2mm diameter foci were scattered throughout the hepatic parenchyma. The lungs were diffusely mottled red and tan and were slightly wet (congestion and oedema). No other abnormalities were detected on gross examination.

Laboratory Results: Culture of colonic contents produced a scant growth of *E. coli* and an *Enterococcus* species. There was no growth of *Campylobacter* or *Salmonella* spp. Low numbers of coccidial (1+) oocysts were seen on fecal flotation examination. ELISA for *Giardia* and cryptosporidia were negative, as was PCR for *Tritrichomonas fetus.*

Histopathologic Description: Liver: Multifocally disrupting the hepatic parenchyma are low numbers of randomly scattered, variably sized foci of lytic necrosis characterized by loss of normal hepatic architecture and replacement by karyorrhectic debris, eosinophilic fibrillar material (fibrin), scattered erythrocytes and occasional degenerate neutrophils. Hepatocytes around the margins of necrotic foci are often swollen with vesicular nuclei and vacuolated cytoplasm (degenerate). Moderate numbers of neutrophils, macrophages and fewer lymphocytes separate foci of necrosis from the surrounding normal hepatic parenchyma. Smaller foci of similar inflammatory cells without significant central necrosis are also scattered throughout. Faintly visible, slender, 4-20 μm long bacilli are rarely visible arranged in sheaves and stacks within degenerate and intact hepatocytes surrounding foci of inflammation and necrosis.

PROXIMAL COLON: The mucosa is multifocally eroded, with affected areas overlain by mats of bacteria admixed with karyorrhectic debris, mucin and degenerate neutrophils. Enterocytes are multifocally hypereosinophilic, rounded up and dissociated with pyknotic nuclei (necrosis). There is diffuse goblet cell hyperplasia. Colonic crypts contain excess mucin within which large numbers of faintly basophilic bacilli are often visible. In addition crypts multifocally contain karyorrhectic debris and degenerate neutrophils (crypt abscesses). Crypt enterocytes are multifocally crowded, with slightly basophilic cytoplasm, vesicular nuclei and increased mitotic figures (regeneration). Moderate numbers of neutrophils and macrophages and fewer lymphocytes and plasma cells diffusely expand the lamina propria, multifocally extend into the submucosa, and rarely infiltrate the tunica muscularis.



1-4. Colon, kitten: Clusters of C. piliforme are present within glandular epithelium lining crypt abscesses. (Warthin-Starry 4.0, 600X)

OTHER TISSUES (not present on submitted slides): Similar, but less severe lesions to those in the colon are present in sections of ileum. A small focus of myocardial necrosis and neutrophil infiltration disrupts the left ventricular myocardium. Alveoli within the lungs diffusely contain lightly eosinophilic homogeneous material (oedema).

SPECIAL STAINS: Low to moderate numbers of slender, silver positive, 4-20 μm long bacilli arranged in criss-crossed stacks are visible within hepatocytes surrounding foci of necrosis in the liver on Warthin-Starry stained sections. The bacteria are difficult to discern with Gram stain. Myriad silver positive, 4-20μm long bacilli arranged in sheaves and crisscrossed stacks are visible within colonic crypts and within crypt enterocytes, and occasionally within the lamina propria on Warthin-Starry stained sections.

Contributor's Morphologic Diagnosis:

LIVER: Moderate, subacute, multifocal, necrotizing and suppurative hepatitis with intracellular argyrophilic bacilli.

COLON: Severe, subacute, multifocal, necrotizing and suppurative colitis with crypt abscesses, goblet cell hyperplasia and myriad intralesional argyrophilic bacilli.

Contributor's Comment: Based on the characteristic lesions in the liver, intestinal tract and heart and intralesional argyrophilic bacteria, Tyzzer's disease was diagnosed as the cause of death in this kitten.

Tyzzer's disease is a commonly fatal disease of primarily young animals caused by the gram-negative anaerobe *Clostridium piliforme*. Spontaneous disease is reported in a range of wild[9,10] and domestic[4,5,6] species, but is particularly well-recognized in laboratory animals (mice, rats, rabbits, gerbils, hamsters and guinea pigs)[8] and foals.[1] Rare cases are reported in cats.[4,7] Disease usually occurs as isolated cases or epizootics with low morbidity and high mortality. Antemortem clinicopathologic findings vary between species. Reports in kittens describe diarrhea, emaciation and depression,[4] while foals developed lethargy, recumbency, fever and seizures, metabolic acidosis, hypoglycemia and increased liver enzymes.[1] Definitive diagnosis is often possible based on characteristic gross and histologic lesions, with silver-positive bacterial rods visible in histological sections of affected liver. Serological tests, PCR and EM have also been utilized in the diagnosis.[1,4,5,9] The organism is extremely fastidious and culture is unrewarding.[7]

Among rodents, B6 mice are resistant to infection compared to CBA/N and DBA/2 mice, and B-lymphocyte function appears to be particularly important in disease resistance.[8] Infected rats may develop a characteristic megaloileitis.[8] Gerbils are recognized to be highly susceptible to Tyzzer's disease and are a useful sentinel animal to detect its presence in research facilities.[8] In rabbits, outbreaks are characterized by profuse diarrhea and high mortality among weanlings. Surviving animals may develop stenosis of the intestinal tract.[8]

The pathogenesis of Tyzzer's disease is only partially elucidated. It is thought that rodents and rabbits may be an important source of infection through shedding of spores in feces.[8,12] The spores may remain infective in contaminated environments for up to a year.[8] Ingested spores are carried through the digestive tract into the small intestine and are able to penetrate mucosal epithelial cells and replicate within them.[12] Bacteria may reach the liver by penetrating capillaries within the lamina propria that drain into the portal venous system, or within macrophages (leukocyte trafficking).[12] Once in the liver, the bacteria penetrate sinusoidal

endothelial cells and adjacent hepatocytes and replicate within them.[12] Lysis of infected cells results in the characteristic areas of lytic necrosis, which may be seen grossly as multiple 2-3mm yellow-grey foci scattered throughout the hepatic parenchyma.[8,12]

Predisposing factors are an important consideration and are frequently reported in cases of Tyzzer's disease.[3,4,6,8] Stress associated with weaning, overpopulation, poor hygiene, immunosuppression due to disease or corticosteroid treatment, and concurrent infections are possible contributing factors.[8] Kittens with Tyzzer's disease in one report were co-infected with feline panleukopenia virus.[4] In reports of Tyzzer's disease in dogs, previous or concurrent infection with distemper, parvovirus and coccidia were described and likely contributed to susceptibility to Tyzzer's.[3,6]

No significant concurrent disease processes were noted in the present case, but the history of diarrhea and recovery in other kittens from the pet store suggest the possibility of an underlying infectious enteritis that was not detected by routine diagnostic screening. The stress of transport and re-homing, housing conditions and access to rodent feces may also have been factors in disease susceptibility, as kittens came from various sources and were housed in cages adjacent to rodents and rabbits. The two other kittens from the pet store that died "suddenly" may also have had Tyzzer's disease; however, no diagnostics were undertaken on those kittens, so other causes of death cannot be ruled out.

Prevention of death from Tyzzer's disease involves maximizing nutritional status, maintaining adequate hygiene of rearing areas, vaccination to prevent predisposing disease conditions, controlling wild rodents, management of parasite burdens, and prompt intensive care of affected individuals.[1,8]

JPC Morphologic Diagnosis: 1. Liver: Hepatitis, necrotizing, multifocal, random, with numerous intracytoplasmic filamentous bacilli. 2. Colon: Colitis, necrotizing, diffuse, moderate, with mild mucosal hyperplasia and numerous intracytoplasmic filamentous bacilli.

Conference Comment: The contributor does an excellent job of highlighting important characteristics of Tyzzer's disease, a diagnosis more commonly assigned to rodents or foals. The liver is the principle target of the bacteria with hepatic necrosis evident regardless of species. Intestinal involvement is variable, but colitis with crypt abscesses is a common component with this disease in cats.[2] Conference participants briefly discussed the merits of using the term "crypt abscess" in the colon, with most favoring it as an accurate term throughout the intestinal tract albeit with the reminder that it actually refers to exfoliated epithelial cells admixed with fewer inflammatory cells.[2]

Necrotizing colitis in kittens is much more common than the occurrence of Tyzzer's disease in this species, thus participants also reviewed the more typical etiologies often associated with this lesion. *Tritrichomonas foetus* causes persistent large-bowel diarrhea in cats less than 1 year of age, with chronic colitis and variably apparent crescent-shaped organisms being the corresponding histologic findings.[2] Although feline panleukopenia virus (FPV, feline parvovirus) more commonly causes small intestinal disease, colonic lesions may also be involved. Feline leukemia virus can cause cryptal necrosis similar to FPV. The small gram-negative bacteria, *Anaerobiospirillum* is associated with ileocolitis in cats in which it causes crypt abscesses and may lead to septicemia or renal failure.[2]

Contributing Institution: Massey University, New Zealand

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CASE II: B2944 (JPC 4041409)

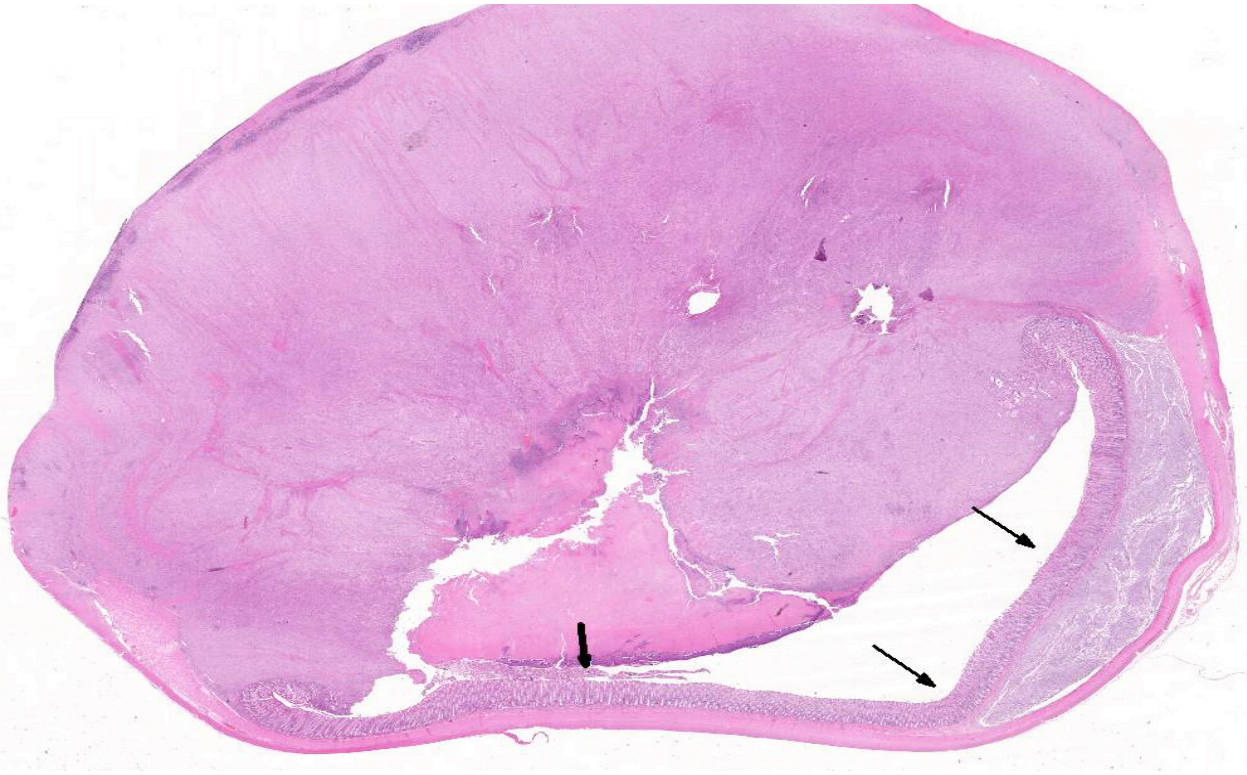
Signalment: 9-year-old neutered male domestic short hair feline (*Felis cattus*).

History: This cat presented with a chronic history of inappetence, lethargy, weight loss and inappropriate defecation. Physical examination revealed a thin cat and abdominal palpation identified a firm, irregular mass present in the caudal abdomen. Biochemistry and hematology samples were obtained and analyzed but were largely unremarkable. Abdominal ultrasonographic evaluation was performed which revealed a large mass in the descending colon. The colonic mass was identified and resected during exploratory laparotomy. Fine needle aspirates of the enlarged mesenteric lymph nodes adjacent to the mass revealed a mixed cellular population consistent with a reactive lymph node. The surgery and recovery period went well without any complications.

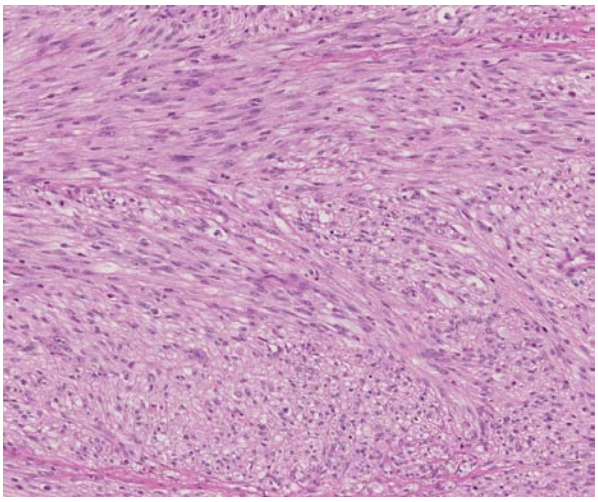
Gross Pathology: A solid mass measuring 2.5 x 3.5 x 1.5cm focally expanded the colonic wall. The cut surface was characterised by firm, white tissue which extended into and partially occluded the colonic lumen.

Laboratory Results: Cobalamin levels were within normal limits, but the folate levels were slightly decreased, at 8.1 µg/L (reference 13.4-38 µg/L).

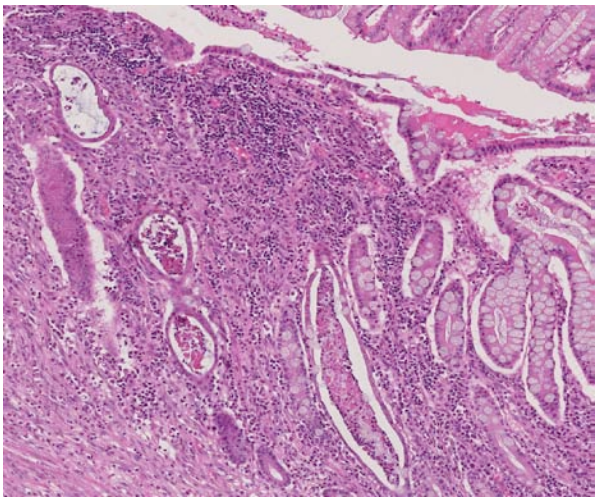
Histopathologic Description: Colon: An unencapsulated, moderately cellular, infiltrative, exophytic mesenchymal mass markedly expands and effaces the mucosa and submucosa, compressing adjacent mucosa and extending into and largely occluding the colonic lumen. Multifocally, the neoplasm infiltrates and destroys the underlying tunica muscularis and extends to the serosa. The neoplastic fusiform or polygonal cells are arranged in sheets and interlacing streams and bundles supported by a fibrovascular stroma. The cells have indistinct borders and contain moderate amounts of eosinophilic fibrillar cytoplasm. They contain a single oval to elongate nucleus, finely stippled chromatin and 1-2 distinct nucleoli. Six mitotic figures are observed in 10 HPFs. There is moderate anisocytosis, moderate to marked anisokaryosis and moderate cellular pleomorphism. Occasional karyomegalic cells are observed. The surface of the exophytic mass is widely ulcerated and replaced by abundant fibrin embedded with necrotic debris, degenerating neutrophils and myriad bacterial colonies.



2-1. Colon, cat: The colon is asymmetrically and transmurally expanded and effaced by a large infiltrative mesenchymal neoplasm. (HE 3X)



2-2. Colon, cat: Neoplastic cells are spindled and elongate, and arranged in broad streams and bundles on a fine fibrous matrix. (HE 116X)



2-3. Colon, cat: The neoplasm infiltrates and replaces the mucosa, and remaining glands are dilated and filled with necrotic debris (crypt abscesses). (HE 77X)

Multifocally, small to moderate nodular aggregates of lymphocytes border the neoplastic mass.

The neoplastic cells did not express alpha smooth muscle actin. Additional tissue sections were immunohistochemically stained for CD117 (c-KIT) and revealed scattered neoplastic cells exhibiting weak to moderate CD117 (c-KIT) positivity.

Contributor's Morphologic Diagnosis: Colon: Gastrointestinal stromal tumor, intermediate to high grade.

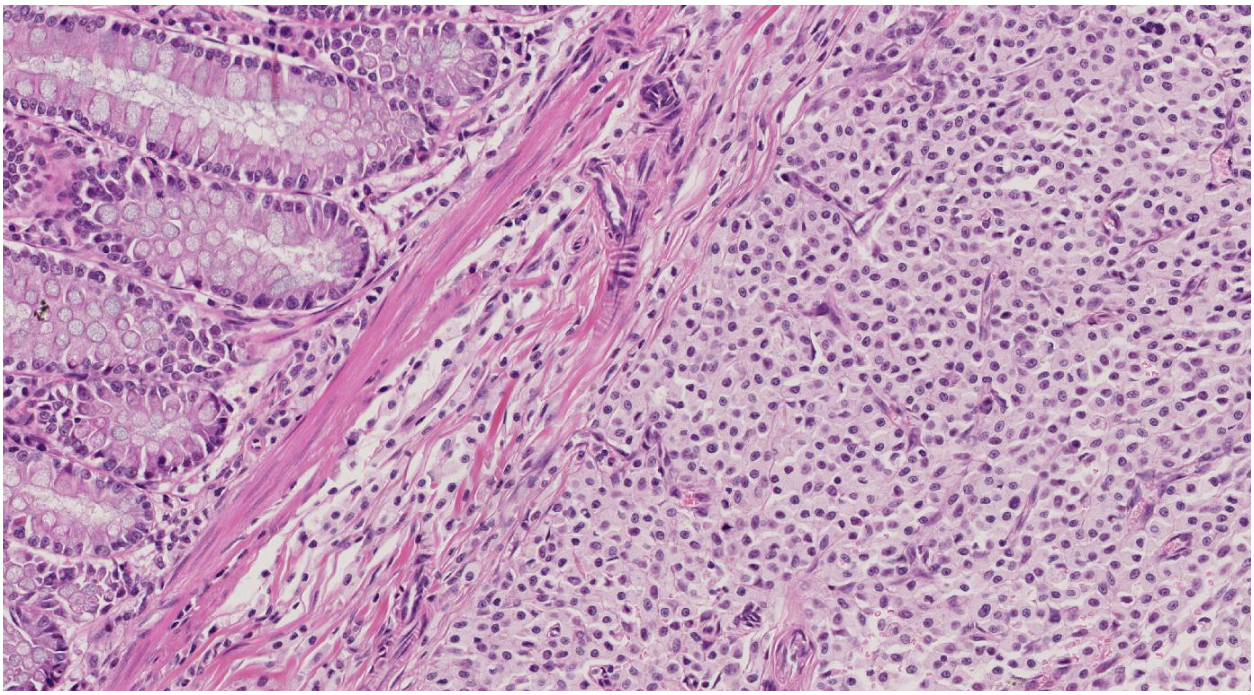
Contributor's Comment: Lymphoma and mast cell tumors are the most frequently encountered types of haematopoietic neoplasia in the feline intestine and adenocarcinomas are the most commonly described non-hematopoietic neoplasm.[9,13] Gastrointestinal stromal tumors (GISTs) account for the majority of mesenchymal tumors in the human gastrointestinal tract.[6,13] They have been previously reported in the dog, ferret, horse and nonhuman primate, however, GISTs are rarely described in cats.[13,6,7]

GISTs are described as a distinctive group of primary mesenchymal gastrointestinal tumors assumed to be derived from the interstitial cells of Cajal (ICC) or their progenitor stem cells.[12] Immunohistochemistry has enabled their recognition as a unique group of gastrointestinal mesenchymal tumours by their cytoplasmic immunoreactivity for c-KIT protein (CD117).[12]

CD117 (KIT) protein is a transmembrane growth factor receptor belonging to the tyrosine kinase family whose ligand is the stem cell factor.[6,13] In the gastrointestinal tract immunohistochemical identification of the CD117 antigen is consistently demonstrable in the ICC (myofibroblasts). These cells are the autonomic nerve-related GI pacemaker cells dispersed between the intestinal smooth muscle cells that co-ordinate intestinal motility and peristalsis.[4,12,13]

Prior to their immunohistochemical characterization, GISTs were classified as leiomyomas, leiomyosarcomas, leioblastomas or schwannomas based on their histological appearance and apparent origin from the tunica muscularis.[13] It is now suggested that these ICC are the histogenic origin of the neoplastic mesenchymal cells in GISTs due to their immunohistochemical similarity producing diffuse strong cytoplasmic expression of CD117.[13,6] The variability in histologic appearance is likely due to different pathways of differentiation within these tumours.

GISTs are more frequently observed in the intestinal tract of the veterinary companion animals, in particular the cecum in horses in comparison to humans where the stomach is the most common site of origin.[4,6,7,13] In a prior study in dogs, GISTs occurred in older animals with no overt sex predisposition and a relatively low incidence of metastasis.[5]



2-4. Colon, cat: A second neoplasm composed of well-differentiated mast cells expands the submucosa. (HE 164X)

In humans, GISTs tend to be more densely cellular than smooth muscle tumors and several histological patterns have been identified of which the most commonly described is the spindloid pattern composed of fusiform cells arranged in whorls or interlacing fascicles or whorls with elongated or cigar-shaped nuclei with perinuclear vacuoles.[14] Less commonly observed is the polygonal or epithelioid variant.[14]

These tumors can express CD34 and SMA in addition to the more consistent expression of CD117 and vimentin.[7] Desmin and S-100 are usually not expressed.[13] In contrast leiomyomas are typically positive for SMA and desmin which are markers of smooth muscle differentiation. These tumors do not express CD117 and usually have an indolent course.[6] Tumors that do not express SMA or CD117 have been described as GIST-like tumors.[11,12]

Prior reports of canine, equine and feline GISTs are histologically and immunohistochemically comparable with the human spindloid variant.[4,13] Smooth muscle differentiation resulting in the co-expression of SMA in some tumours supports the hypothesis that GISTs develop from a pluripotential ICC or its progenitor cell type which can differentiate into smooth muscle cells.[4,13]

Alteration of the gene encoding the tyrosine kinase receptor KIT is considered an early molecular event in the development of GISTs.[13] This enables ligand-independent receptor activation and subsequent signal transduction leading to cellular proliferation.[6] In human, canine and feline GISTs the majority of mutations have been identified in the juxtamembrane domain (exon 11) of the gene.[6,13] These KIT mutations are potential targets for therapeutic invention with tyrosine kinase inhibitors.[13]

It is difficult to determine the degree of malignancy of GISTs as their biological behaviour is not well characterised in animals.[13] In this case, following diagnosis, the cat was treated with toceranib phosphate (Palladia) for 10 months with no recurrence or metastatic spread of the tumor 2 years after surgical resection.

JPC Morphologic Diagnosis: 1. Colon: Gastrointestinal stromal tumor. 2. Colon: Mast cell tumor, well-differentiated.

Conference Comment: As occasionally occurs with WSC submissions, the serial sectioning of 180+ slides may uncover distinctive pathologic processes which were not originally identified by the contributor. In this example, some conference participants had, in addition to the described

GIST, a well-circumscribed, densely cellular neoplasm composed of round cells which separates the muscular layers of the colon from the submucosa. The neoplastic cells have faintly granular cytoplasm, a centrally-placed nucleus, and stain multifocally for CD117 which is most indicative of a mast cell tumor. As the contributor outlines, mast cell tumors are quite common within the intestine of cats, accounting for the third most frequently encountered neoplasm within this location.[10]

The contributor thoroughly discusses the immunohistochemical characteristics of GISTs, which became important in their differentiation from tumors of muscle origin. Although these are typically discovered as incidental findings at necropsy[4], this case portrays the magnitude to which these tumors can grow and cause functional obstructions as observed clinically in this cat. They have also been reported to induce paraneoplastic hypoglycemia by their production of insulin-like growth factors and erythrocytosis from their production of erythropoietin.[3]

An abdominal mass in the cat must also include the non-neoplastic lesion, eosinophilic sclerosing fibroplasia, as a possible differential which may also lead to obstructive clinical signs. This mass of eosinophils, fibroblasts and collagen may be found throughout the gastrointestinal tract in cats of all ages. It is hypothesized this is an inflammatory response to a bacteria and may be related to the cat's affection for recruiting eosinophils in other dermatologic and oral conditions known collectively as the eosinophilic granuloma complex.[5]

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CASE III: 12-8651 (JPC 4032480)

Signalment: 4-year-old intact male miniature Schnauzer dog *(Canis familiaris)*.

History: The dog had experienced some weight loss in the past several months. Firm nodules were palpated on the testicles. The referring veterinarian submitted the testicles to rule out infection and neoplasia.

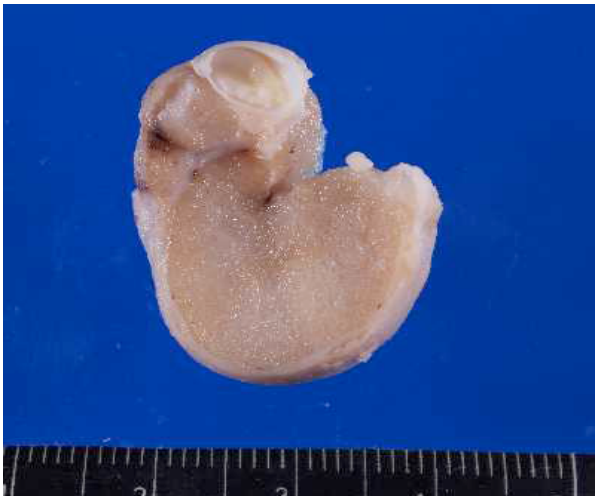
Gross Pathology: At castration surgery the testes and epididymis were thickened by white material. Both testicles were submitted for histopathology. At sectioning, multiple cystic nodules were present within the tunics.

Laboratory Results: None.

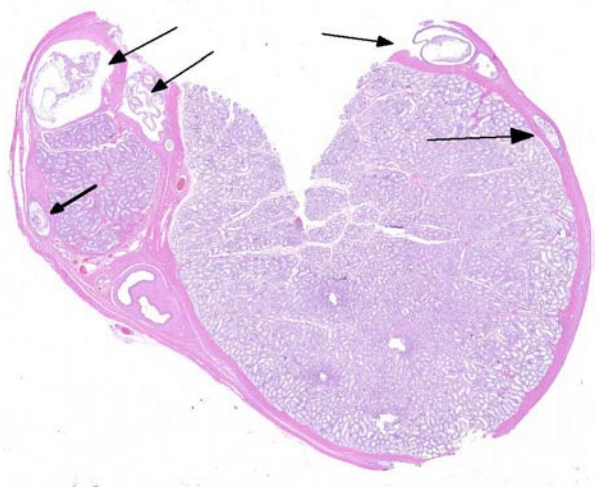
Histopathologic Description: Both testicles were equally affected. The testicular cords (between the pampiniform plexus and spermatic duct), tunica albuginea and the stroma of the epididymis were multifocally expanded by many cross sections of variably sized and variably degenerate acoelomate larvae within cystic spaces lined by either streams of fibrous connective tissue or a thick bands of inflammatory cells. Cestode larvae ranged from 1,000 x 600 µm to 1,600 to 900 µm and had thick (45 µm), convoluted hyaline cuticles overlying single layers of palisading epithelial cells. Within the loose mesenchymal parenchyma digestive tracts were not observed, and there were numerous, 50 x 40 µm, oval to pleomorphic, clear vesicles with widely scattered, small, weakly basophilic concretions (calcareous corpuscles). No scolices were observed. Inflammatory infiltrates flanking parasites were composed of moderate numbers of lymphocytes, plasma cells, epithelioid macrophages, neutrophils and eosinophils; granulocytes were predominantly within areas of degenerating larvae. Within the testicular parenchyma spermatogenesis was present; epididymal ducts were histologically within normal limits and contained some sperm.

Contributor's Morphologic Diagnosis: Periorchitis, epididymitis and funiculitis, lymphohistiocytic and pyogranulomatous, severe, bilateral and multifocal, chronic with intralesional cestode larvae (most likely *Mesocestoides* sp.).

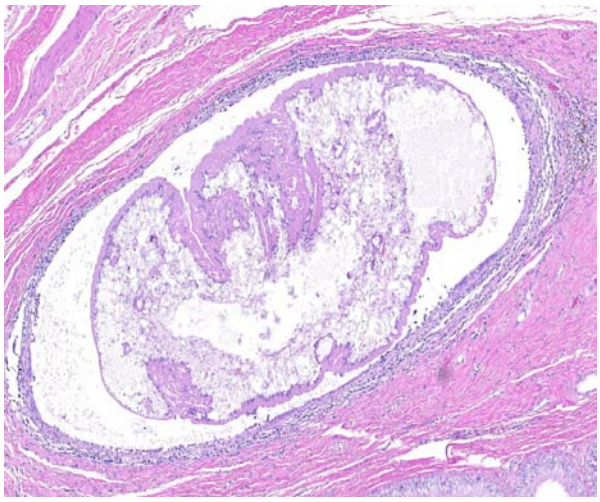
Contributor's Comment: Mesocestoides spp. are tapeworm parasites with unusual 3 host life cycles.[7] The first intermediate hosts are purported to be coprophagous insects, which are ingested by a variety of small reptiles and rodents. Within the second intermediate host, the metacestode, called a tetrathyridium, penetrates the intestinal wall and undergoes asexual replication within the peritoneal cavity. Ingestion of the second host by the definitive host completes the life cycle; infection with adult *Mesocestoides* are largely asymptomatic. Proglottids from adult parasites are not directly infectious to definitive or secondary intermediate host species.[7] Definitive hosts are mainly wild and domestic canids, although



3-1. Testis, dog: At sectioning, multiple cystic nodules were present within the vaginal tunics. (Photo courtesy of: Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-7040, www.vetmed.wsu.edu)



3-2. Testis, dog: Within the vaginal tunics, there are numerous cross sections of degenerating cestode parasites. (HE 0.63X)



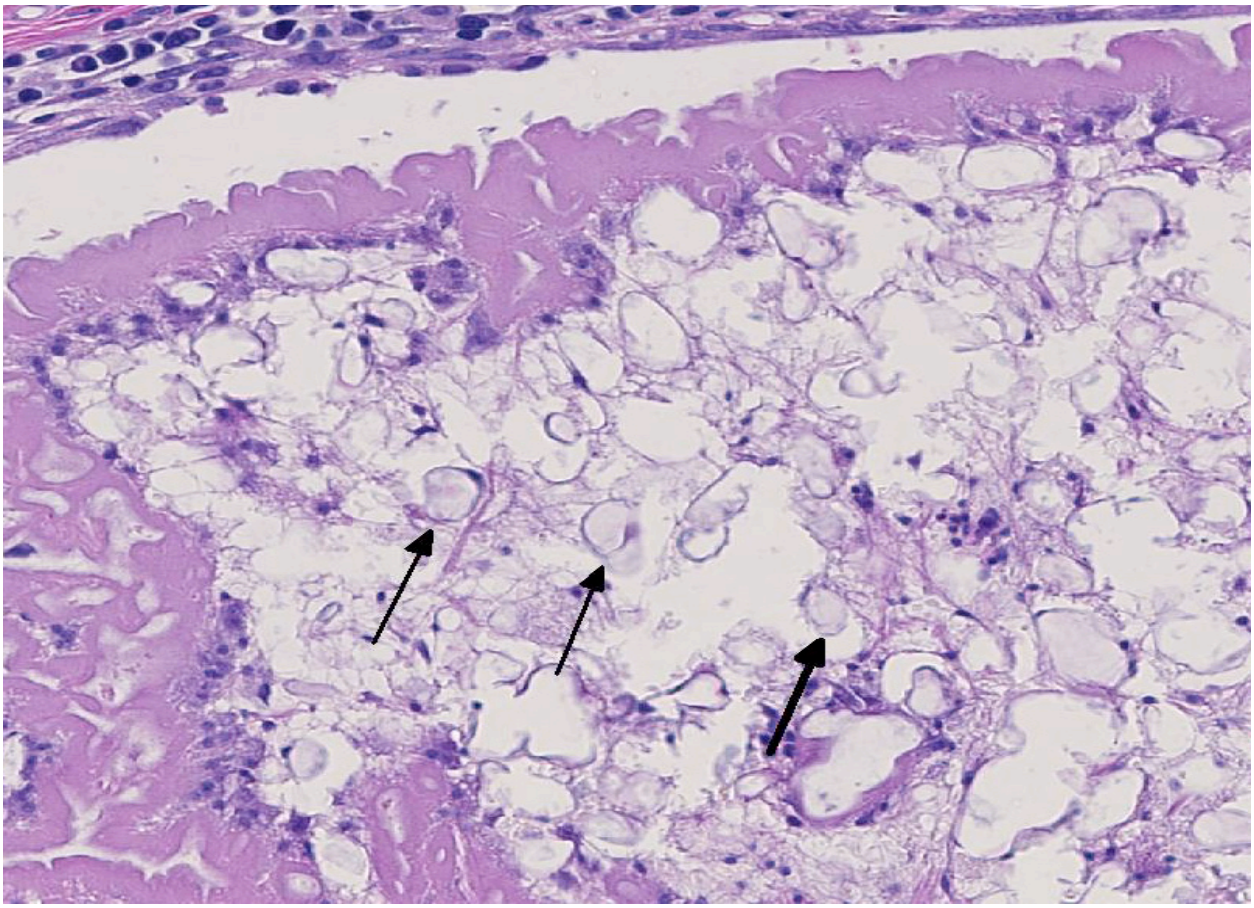
3-3. Testis, dog: Degenerating cestode paraasites are surrounded by a dense fibrous capsule which is lined by epithelioid macrophages, lymphocytes, and plasma cells. (HE 120X)

multiple species, including humans[5], can become infected. Distribution of the parasites is worldwide. Veterinary interest in the species is largely related to rare migration of ingested tetrathyridia into the abdominal cavity of domestic dogs and occasionally cats.[12] The subsequent prolific asexual reproduction of metacestodes and the ensuing severe granulomatous peritonitis has been termed canine peritoneal larval cestodiasis (CPLC).[2] In rare cases, parasites extend into abdominal organs and into the thoracic cavity.[11] It is not known whether CPLC truly represents aberrant migration of tetrathyridia through the intestinal wall after ingestion of the second intermediate host, or if it represents aberrant use of the dog as a secondary intermediate host after accidental ingestion of the first intermediate host.[2]

Diagnosis and treatment of CPLC can be challenging. Clinical signs are vague and include lethargy, weight loss, vomiting and ascites.[4] Occasionally subclinical cases are identified during routine ovariohysterectomy or castration.[2] In two reported cases, scrotal swelling was among the initial presenting complaints.[10,13] Diagnostic procedures include radiology, showing changes indicative of diffuse peritonitis, and ultrasonography.[12] Cytologic examination of the ascites fluid or aspiration of affected organs often reveals intact tetrathyridia, acephalic forms or calcareous corpuscles.[3,9] Diagnosis of infection by *Mesocestoides* spp. can be confirmed by morphologic identification of tetrathyridia or by PCR.[2] Recommended treatment involves

COMMON VETERINARY CESTODE SPECIES		
ADULT	**LARVA**	**SPECIES WITH ASSOCIATED PATHOLOGY**
Spirometra spp.	*Sparganum (Plerocercoid)*	Dog, Cat
Mesocestoides spp.	*Tetrathyridium*	Dog, Cat, NHPs
Rodentolepis nana, R. microstoma, Hymenolepis diminuta	*Cysticercus*	Rodent
Taenia taeniaformis	*Cysticercus fasciolaris*	Rodent
Taenia pisiformis	*Cysticercus pisiformis*	Rabbit, Dog
Taenia hydatigena	*Cysticercus tenuicollis*	Ruminants, Swine
Taenia ovis	*Cysticercus ovis*	Sheep
Taenia saginata	*Cysticercus bovis*	Cattle
Taenia solium	*Cysticercus cellulosae*	Swine
Taenia multiceps	*Coenurus cerebralis*	Sheep, Goat
Moniezia spp., Thysaniezia giardi, Stilesia globipunctata	*Cysticercus*	Ruminant
Anoplocephala perfoliata	*Cysticercus*	Horse
Diphyllobothrium spp.	*Cysticercus*	Fish-eating carnivore
Diplydium caninum	*Cysticercus*	Dog, Cat
Echinococcus granulosus	*Hydatid cysts*	Many
Echinococcus multilocularis	*Hydatid cysts*	Many

peritoneal lavage and long term treatment with fenbendazole[4] although other drugs, such as praziquantel, have also been used.[8] Prognosis after treatment depends upon the severity of infection at the time of diagnosis and upon how aggressively therapy is instituted.[2] At least some dogs experience recrudescence months after therapy is discontinued, although reinfection cannot be ruled out.[1] The dog in this report had a massive peritoneal infection and was treated aggressively with lavage and 30 days of fenbendazole therapy. Four months after initial diagnosis he presented with vomiting and radiographic evidence of intestinal obstruction. Partial necropsy after euthanasia revealed massive abdominal adhesions, but no samples for histopathology were collected; whether adhesions



3-4. Testis dog: Degenerating cestodes have a thick, smooth tegument, subjacent layer of somatic cells, a spongy parenchymatous body cavity, with numerous calcareous corpuscles (arrows). (HE 284X)

represented complications of healing peritonitis or recurrence of cestodiasis was not determined.

Although PCR to confirm *Mesocestoides* infection was not performed in this case, histologic lesions were consistent with previously reported cases. Characteristics of cestodes include a thick cuticle, acoelomate body and numerous calcareous corpuscles.[6] Although most of the metacestodes were degenerate, examination of multiple sections failed to reveal definitive scolices or suckers; it is likely, therefore that most of the organisms in this dog were the acephalic forms. Both tetrathyridia and acephalic forms have been described in cases of CPLC.[4]

JPC Morphologic Diagnosis: Testes, vaginal tunic: Larval cestodes, multiple, with mild granulomatous periorchitis.

Conference Comment: With the poor preservation of the larval cestodes in most sections, conference participants struggled to assign a specific etiologic diagnosis to this case. The presence of calcareous corpuscles indicates that the parasites are cestodes, few species of which are present specifically in the vaginal tunics, an extension of the peritoneum. R. *Spirometra* spp. is a cestode which may encyst in the peritoneal cavity of carnivores. Its larval form, sparganum, is also solid-bodied and only differentiated from the tetrathyridium of *Mesocestoides* spp. by its lack of suckers or scolex.[6] Obtaining definitive evidence of suckers or a scolex requires serial sections through a well-preserved organism, thus we favored the diagnosis of proliferating larval cestodes in this example.

The class of flatworms (*Platyhelminthes*) referred to as cestodes are more commonly known as tapeworms, and infect nearly every vertebrate species. Cestodes typically inhabit the gastrointestinal tract, to include the ducts of the liver and pancreas. They are generally of minor significance to the animal though they may reach

spectacular size in some species (Consider the 60-foot long, *Diphyllobothrium latum* of man!). All tapeworms utilize at least two hosts to complete their lifecycle, and as the contributor concisely describes, some such as *Mesocestoides* spp. require more.

Adult cestodes are segmented into proglottids and both larvae and adults often contain suckers or hooks. All contain calcareous corpuscles which are of unknown function but most helpful in initially classifying the organism as a cestode. In this case, the corpuscles are shrunken and surrounded by a clear space which is likely a commonly observed artifact of fixations. *Spirometra* falls into the category with *Diphyllobothrium* of Pseudophyllideans. *Mesocestoides* are grouped with other common veterinary parasites such as *Taenia, Hymenolepis,* and *Echinococcus* in the Cyclophyllidean category characterized by four anterior suckers and muscles separating medullary and cortical regions.[6]

Since nearly all adult tapeworms are parasites of the digestive tract, those observed in tissue sections are usually larva. Along with the discussed sparganum and tetrathyridium larvae, most other larval forms have specific nomenclature. Pairing the appropriate larval and adult tapeworm species together can be an overwhelming challenge, though a focus on the following cestodes may cover those of greatest importance in veterinary pathology.

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CASE IV: F1409682 (JPC 4048672)

Signalment: Adult female intact chicken (*Gallus gallus domesticus*), breed unknown.

History: 4-week history of respiratory illness in the flock, affecting up to 35 birds. Clinical signs include coughing and sneezing in a subset of birds. This patient was found deceased.

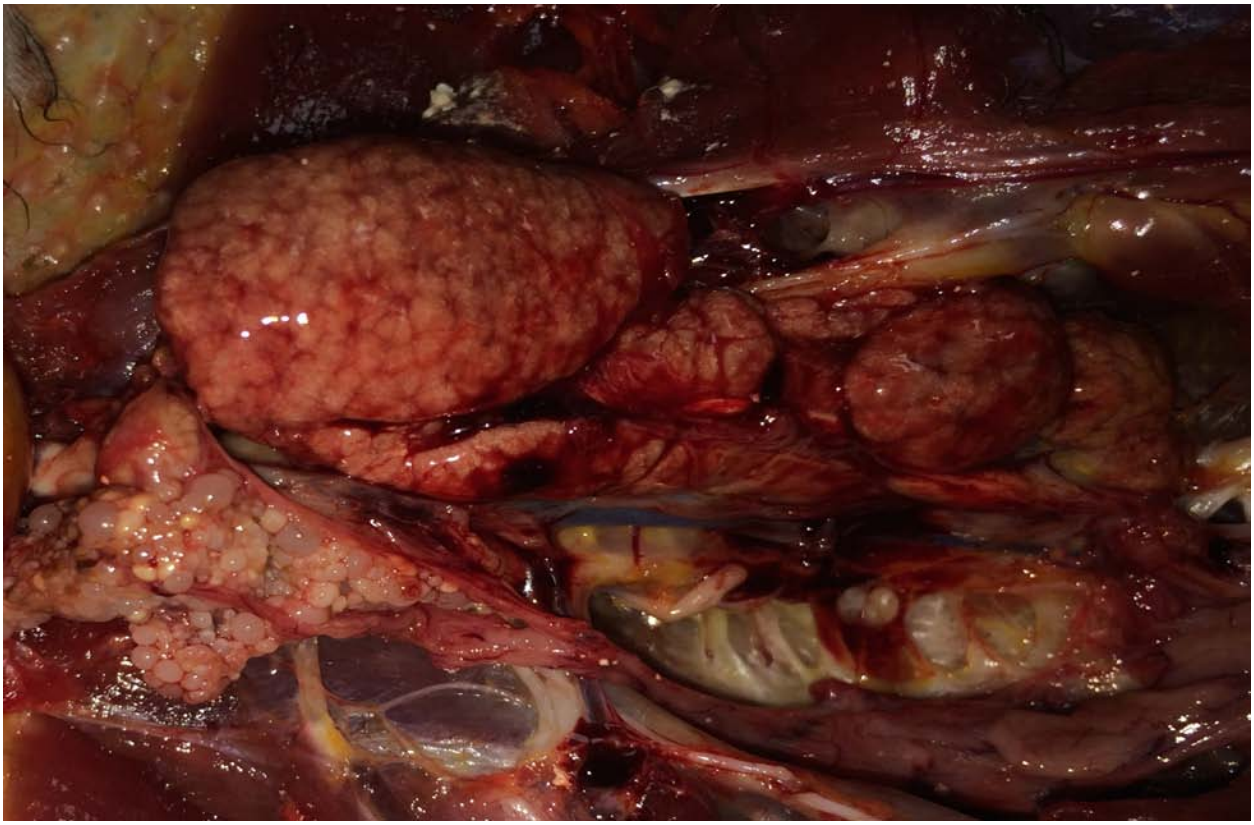
Gross Pathology: A brown hen was submitted for necropsy in poor body condition with a prominent keel due to marked atrophy of the pectoral muscles. There is a minimal amount of internal body fat stores and subcutaneous fat is not present. The kidneys are diffusely enlarged up to 3x normal, mottled pale to red in color, soft and friable, and bulge slightly upon cut section.

Laboratory Results: Tracheal swab Avian Influenza real-time PCR Negative

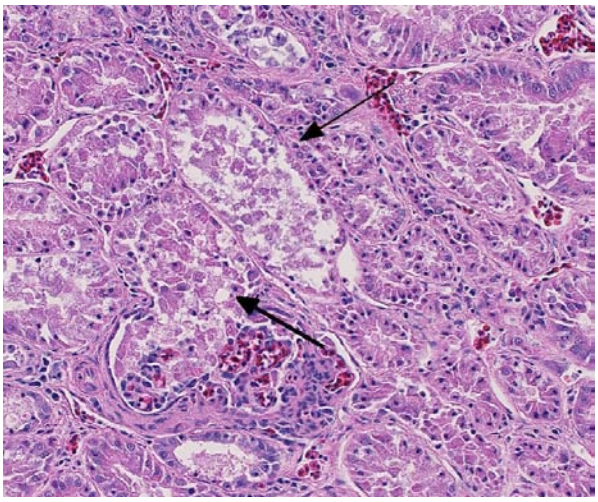
Tracheal swab Infectious Bronchitis Virus real-time PCR Negative

Kidney Infectious Bronchitis Virus real-time PCR Negative

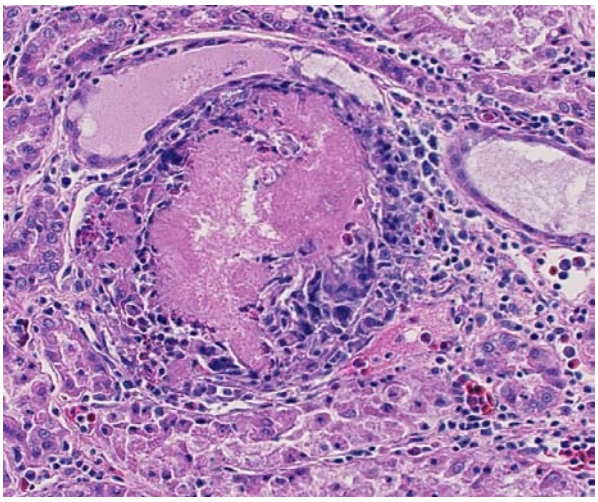
Histopathologic Description: Diffusely throughout the renal parenchyma, there is widespread degeneration and necrosis of the renal tubular epithelium characterized by swollen cells with vacuolated cytoplasm and swollen nuclei (degeneration) or loss of cellular detail, eosinophilic cellular debris and pyknotic nuclei (necrosis). Tubules have segmental loss of epithelium with epithelial attenuation across bare basement membrane. Multifocally, small numbers of tubular epithelial cells contain increased amounts of basophilic cytoplasm, have anisokaryosis and rare mitotic figures (regeneration). Renal tubules are often ectatic, dilated up to 3x normal and contain numerous epithelial cell casts, moderate numbers of heterophils, occasional granular casts and/or amorphous, eosinophilic material (protein). The interstitium is expanded by coalescing aggregates of lymphocytes, plasma cells, and occasional heterophils. This inflammatory infiltrate is accompanied by mild to moderate amounts of



4-1. Kidneys, chicken: The kidneys are diffusely enlarged up to 3x normal, mottled pale to red in color, soft and friable. (Photo courtesy of: Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, http://csu-cvmbs.colostate.edu/academics/mip/Pages/default.aspx)



4-2. Kidney, chicken: There are clusters of necrotic tubules scattered throughout the kidney. (HE 240X)



4-3. Kidney, chicken: Rare gouty tophi, surrounded by foreign body macrophages, are scattered throughout the kidney. (HE 256X)

edema and fibrosis. Additionally, renal tubules and glomeruli within the cortex are multifocally expanded and replaced by sharp, radiating, eosinophilic crystalline deposits that range in size from 50-150 microns in diameter and are surrounded by moderate numbers of degenerate heterophils and macrophages (urate tophi). Rarely, the parietal epithelium of Bowman's capsule is mildly hypertrophic, characterized by plump, cuboidal epithelial cells that protrude into the glomerular space.

Contributor's Morphologic Diagnosis: Kidney: Tubulointerstitial nephritis, diffuse, lymphoplasmacytic and heterophilic, chronic, severe with tubular necrosis, degeneration and regeneration, urate tophi, cellular casts and tubuloproteinosis.

Contributor's Comment: Tubulonephritis in chickens typically occurs as a result of infection with either the nephrogenic strain of infectious bronchitis virus or avian nephritis virus. Infectious bronchitis virus (IBV) is a coronavirus with a non-segmented, positive-sense, single-stranded RNA genome that infects all ages of chickens and results in major economic losses for the poultry industry.[3,5] Clinical manifestations of IBV infection include respiratory disease, reproductive disorders, and nephritis, with the latter presenting as a progression from respiratory illness to clinical signs of renal failure such as excessive water intake, rapid weight loss and diarrhea.[4] Infection with the nephrogenic strain of IBV initially results in viral replication in the trachea, followed by spread to the kidney and infection of the renal tubular epithelium.[1,2,5] Histologic lesions include interstitial polymorphonuclear inflammation, renal tubular degeneration and necrosis, and uric acid precipitation, with tubular regeneration occurring in surviving birds.[1,2,5] Detection of IBV infection is required for a definitive diagnosis, and is most commonly achieved by virus isolation, reverse-transcriptase polymerase chain reaction (RT-PCR), or serology.[3] In this case, RT-PCR was performed directly on a tracheal swab and on RNA extracted from kidney, with negative results. However, as previously demonstrated, IBV antigen is detected up to day 13 post-inoculation by immunohistochemistry, but not after, suggesting clearance of the virus.[1,2] The 4-week span of respiratory illness with progression to nephritis in this case may have allowed sufficient time for clearance of IBV from the trachea and kidney prior to sampling, thus resulting in a negative result on RT-PCR. Furthermore, if the primers used in the RT-PCR assay are not from conserved regions across all IBV strains, false negatives may occur.[3] Tracheal or renal clinical samples may also can contain non-specific inhibitors of the polymerase used in PCR, resulting in decreased sensitivity.[3] Together, these points highlight the difficult challenge of obtaining a definitive diagnosis of IBV infection, even in the presence of strongly supportive clinical signs and histopathologic findings. The clinical history of respiratory illness in this flock, together with the gross and histologic lesions observed in this adult chicken, are consistent with nephrogenic IBV infection. These findings

underscore the importance of recognizing distinct clinical signs and histopathological lesions so that an accurate diagnosis is made, allowing for the institution of appropriate flock management.

Alternatively, avian nephritis virus (ANV) represents another possible differential for the renal lesions observed in this case. Avian nephritis virus is a non-enveloped astrovirus with a single stranded, positive sense RNA genome that affects young poultry and results in degeneration of the renal tubules and interstitial inflammation, with formation of lymphoid follicles in later stages.[6,8,9] Virus isolation, serology, and/or RT-PCR are the most common laboratory methods of detecting ANV and a positive result is required for definitive diagnosis.[10] Although renal lesions in this case may be consistent with ANV infection, this virus is not documented to cause respiratory disease, such as that observed in this bird and the rest of the flock. Additionally, lymphoid follicles seen in chronic cases of ANV infection were not observed in the kidney of this case, despite 4-week duration of disease.

JPC Morphologic Diagnosis: 1. Kidney, tubules: Degeneration, necrosis, and regeneration, multifocal, moderate, with intratubular protein. 2. Kidney, cortex: Gouty tophi, multiple.

Conference Comment: The contributor provides an overview of the two most likely etiologic differentials in this case, and conference participants, whom are not privy to the provided clinical history, added avian influenza and Newcastle disease as well. Herpesvirus and polyomavirus may each also cause tubular necrosis in avian species, however, intranuclear inclusions would be prominent in both instances.[4]

Much of the conference discussion was focused on whether the gouty tophi and the described concentric amorphous structures, interpreted by most to be protein, were associated with the infection. Urates are actively excreted by renal tubular epithelium, so it stands to reason that loss of tubular epithelium can lead to urate accumulation and subsequent tophi formation. Additionally, a sick bird as described in this case would likely be dehydrated which will further exacerbate urate deposition. The gouty tophi formation was minimal in most sections, thus not likely a significant contributor to the tubular lesions as observed in cases of true renal gout.

As exemplified here, definitive diagnosis with even the ultra-sensitive PCR is challenging with infectious bronchitis virus, the most likely cause of disease in this case. This virus is unique among coronaviruses in that it replicates, mutates and recombines rapidly resulting in the creation of an extensive number of serotypes. These pose a challenge to poultry producers and veterinarians, as vaccines do not provide cross-protection for different serotypes, necessitating the need to develop specific vaccines to the identified serotype associated with an outbreak. Even the vaccines themselves have been associated with the emergence of new strains capable of causing disease. The most significant protein for virus detection is the club-shaped glycoprotein known as spike. Spike mediates cell attachment, fusion and target specificity. It is composed of two subunits: S1, which makes up the outer portion; and S2, which anchors it to the viral envelope. The S1 protein is the common target for RT-PCR and can be used to predict levels of cross-protection between different serotypes of IBV, thus making it the focus of research and vaccine development for this enduring disease.[7]

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