Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

Conference 19

13 February 2019

Signalment: Female, Soft-shelled Turtle (*Apalone ferox*, formerly *Trionyx ferox*), Unknown age (adult)

History: Found dead in an aquarium

Gross Pathology: Multifocal hyperkeratotic and raised circumscribed lesions on the skin of ventral neck and ventral body (plastron) were observed on gross examination. The carcass was found in a fair to poor body condition. Liver was enlarged and pale. Intestines had watery grayish green contents. There was accumulation of hard dry excreta in the distal large intestine (coprostasis). Several developing ova were seen in the coelomic cavity. Stomach was empty. No other grossly visible lesion.

Laboratory results: Several tissues were positive for ranavirus by PCR.

Microscopic Description:
Several myocardial fibers of the sections of heart were disrupted by parasite eggs measuring up to 100x50μm in size. The eggs had 2-3 μm in diameter yellowish refractile often fragmented wall and occasionally contained 2-4 μm in diameter blue round
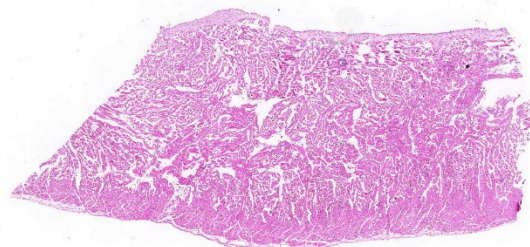
Heart, turtle: A section of ventricular myocardium is submitted for examination. Multiple egg-induced granulomas may be seen from this magnification. The spongy appearance of the myocardium is normal for reptiles. (HE, 5X)

aggregates (miracidium). On the walls of few of these eggs were 5-7 μm in length projecting lateral spines. In some areas, the eggs were surrounded by multinucleated foreign body type giant cells. The eggs were diffusely disseminated in several tissues including the intestines, spleen, liver, kidney and thyroid gland (sections not included). Within vascular lumens in some sections of the heart and liver were about 150 to 300 μm in size adult trematodes. Sections of skin had deep dermal focally extensive areas of necrosis surrounded by moderate amount of collagen fibers occasionally infiltrated by few to several lymphocytes, plasma cells and macrophages. There were no other remarkable lesions.
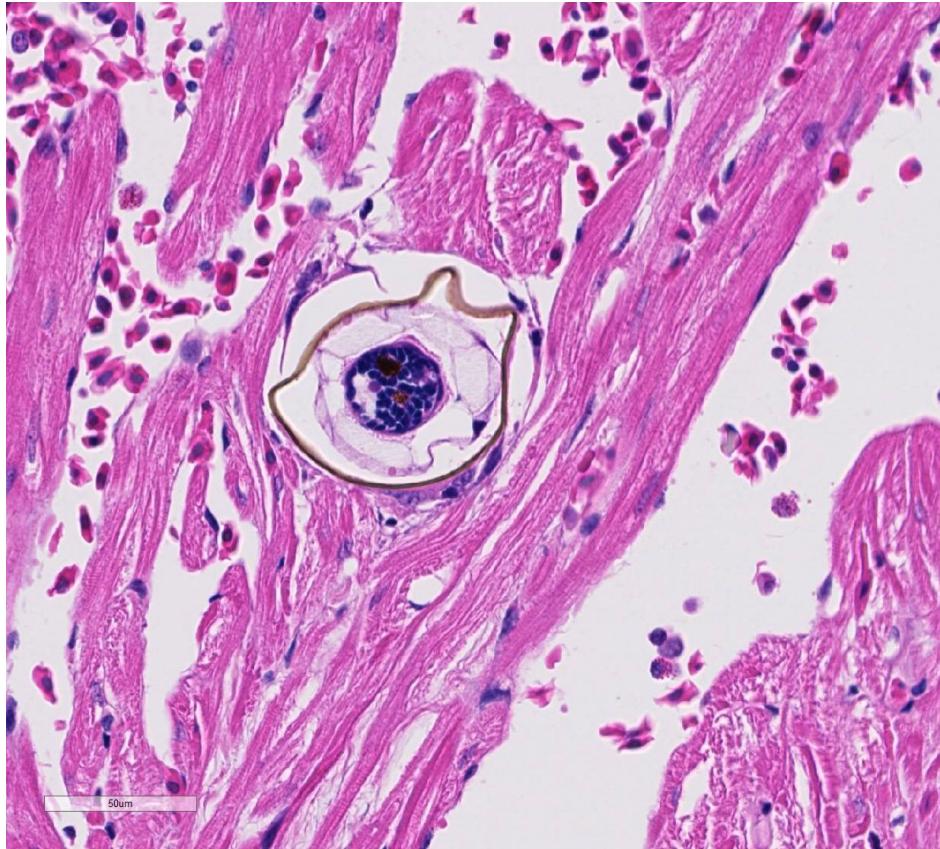
Heart, turtle: Trematode eggs are scatted throughout the myocardium. The eggs have a thin brown shell and a miracidium within. Eggs are surrounded by one or more epithelioid or multinucleated giant cell macrophages. (HE, 400X)

Contributor's Morphologic Diagnoses:
Heart: Myocarditis, Granulomatous, mild to moderate with trematode eggs and occasional intravascular trematodes consistent with Spirorchid sp.

Granuloma, diffuse moderate to marked disseminated in several organs with trematode eggs and intravascular trematodes (spirorchid), Disseminated Spirorchidiasis.

Contributor's Comment: Intravascular trematodes (blood flukes) are reported in mammals, birds, reptiles and fish.[5] Various species of intravascular spirorchid trematodes, including *Learedius learedi, Carettacola hawaiiensis, Hapalotrema dorsopora* and *H. postorchis* are reported in turtles. Specific species identification was not made in the current case. Adult spirorchids inhabit the circulatory system of infected hosts. Eggs may be observed in almost every tissue in the body including the brain, heart, lung, liver, spleen, urinary bladder, shell and slat glands; and may initiate mild to severe granulomatous inflammation. Infected animals may show debilitation, edema of the limbs, and secondary bacterial infections and death. Diagnosis is usually made at necropsy or upon identification of parasites in tissue sections.[2] Serology may be useful in evaluating exposure to the infection, although it may not reflect worm burdons.[5] Microscopic lesions reported in turtles infected with spirorchids include vasculitis (lymphoplasmacytic endarteritis), thrombosis, perivascular hemorrhage, granulomatous cystitis, enteritis, pneumonitis, hepatitis, meningitis, encephalitis, and cirrhosis.[2,4] Remarkable vasculitis was not seen in this case. Coprostasis and poor body condition could be associated to intestinal infection with consequent maldigestion and malabsorption.

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31793,

JPC Diagnosis: Heart, ventricular myocardium: Trematode eggs, intravascular and intrahistiocytic, with mild multifocal cardiomyocyte degeneration.

JPC Comment: The superfamily Schistosomatoidea contains intravascular flukes in three families – Sanguinocolidae, Schistosomatidae, and Spirorchidae.

Schistosomes are grossly visible flukes which cause disease in a wide range of birds and mammals. These parasites have separate genera but the male and female are joined throughout much of their extended life cycle (ranging from 3-30 years). They feed on blood cells and globulins, which are digested in a blind digestive tract with residua regurgitated into the host's circulation.[1] In humans, schistosomiasis (Bilharzia) affects millions of people with up to 200 million on prevention annually.[3] The most widespread and pathogenic human schistosomes are *S.mansoni, S. japonicum,* and *S. haematobium. S. mansoni* and *S. japonicum* live within mesenteric veins, and their eggs are trapped within intestinal walls, resulting in acute bloody diarrhea, and chronic, hepatosplenic granulomatous disease and fibrosis. The eggs of these two flukes are excreted in the feces. *S. haematobium* lives within the venous plexi adjacent to the urinary bladder resulting in urinary bladder ulceration and hematuria, and chronically in proliferative and fibrosis lesion of the urinary bladder, and less commonly, ureters. Its eggs are excreted in the urine.[1]

The family Spirorchiidae, parasites of turtles include 16 recognized genera, and the flukes live within the cardiovascular system. Eggs of these parasites lodge within all organs of the body, forming microgranulomas (as seen
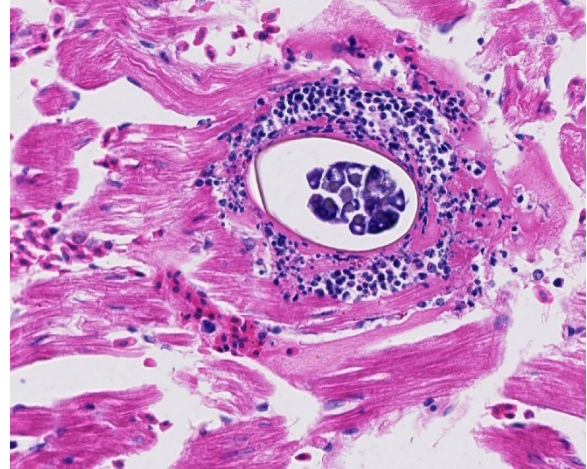

Heart, turtle: Rarely, degenerating eggs incite acute inflammation within the myocardium (HE, 400X)

in this slide.) The life cycle of spirorchid flukes of freshwater turtles involves a pulmonate snail; that of marine turtles is largely unknown, although a common species of blood fluke, *Learedius learedi*, has been identified in the limpet *Fissurella nodosa*.[6]

Of particular current interest is how the lifecycle of blood flukes is completed at all, without direct access of female flukes to intestinal (or in some cases) urinary bladder. Eggs are laid directly into the bloodstream, and form microgranulomas within tissues. Over the last decade, researchers in the area of human schistosomiasis have begun to unravel the way that flukes and their eggs exploit the immune system to get into the external environment.[3] After egg shell components facilitate endothelial adhesion within intestinal vessels, the microgranuloma surrounding the egg actually facilitates the translocation process from intestinal vessels, through the lamina propria and mucosal epithelium and ultimately into the intestinal lumen. The importance of the immune system has been demonstrated by the low fecal egg counts seen in infected immunosuppressed mice; recent research has also focused on the importance of the
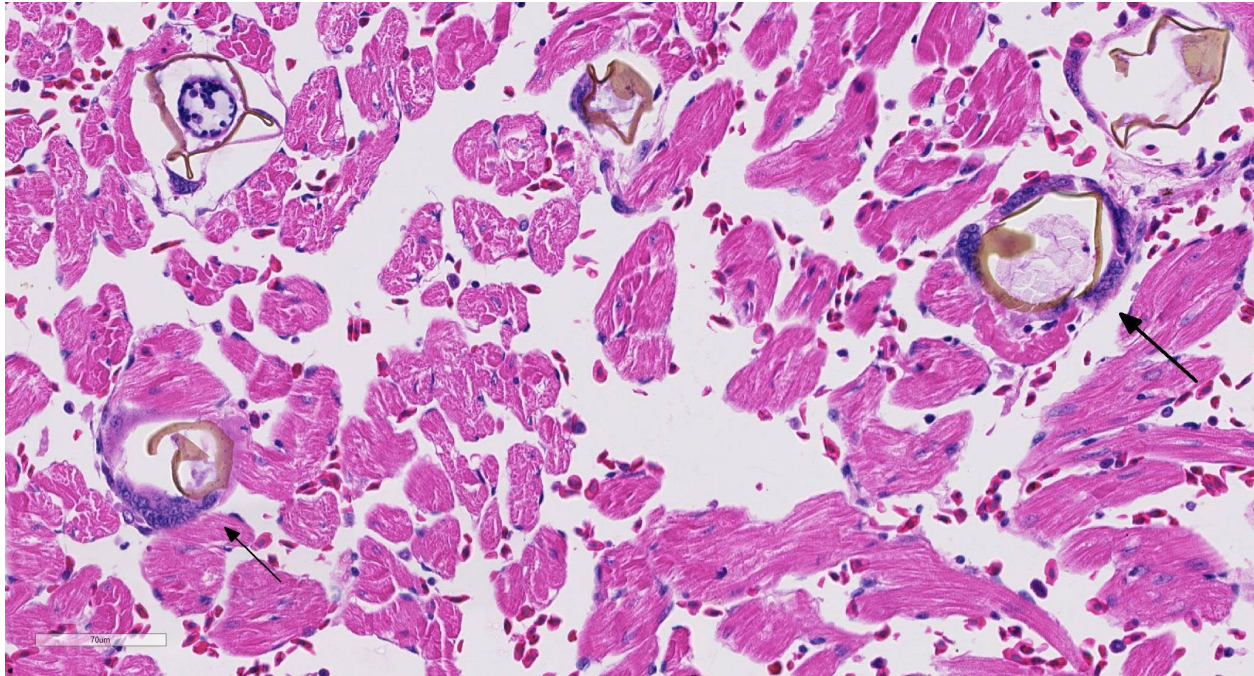
Heart, turtle: Degenerate or fragmented eggs are surrounded by multinucleated foreign body-type macrophages. (HE, 400X)

intestinal microbiome as well. It is likely that a similar process occurs within *Spirorchis*-infected turtles as well.[3]

The moderator discussed an upcoming paper (currently in press) which identifies a fatal outbreak of spirorchid parasites in black pond turtles from Thailand.

References:

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CASE II: TAMU-2 2014 (JPC 4054746).

Signalment: Adult, female, Leopard Tortoise (*Stigmochelys pardalis*)

History: Animal presented to clinic for dyspnea, oral/ocular/nasal discharge, lethargy, anorexia and mucoid cloacal discharge. Eyes matted shut. No fecal/urinary production. This recently purchased tortoise arrived emaciated and

Colon, leopard tortoise. There is diffuse, severe necrotizing colitis. (Photo courtesy of Department of Veterinary Pathobiology, College of Vet Med and Biomedical Sciences, Texas A&M University, College Station, TX USA 77843-4467)

anorectic with oculonasal discharge. Animal had anemia, hyperuricemia, hypercalcemia and hyperkalemia, and renal disease was suspected. Radiographs indicated possible pneumonia. Treated for 3 days, when owner opted for humane euthanasia.

Gross Pathology: The carapace had "pyramiding" indicating metabolicor disease. Other significant gross lesions included: fat pad loss (emaciation), severe enterocolitis, hepatic lipidosis, pleuritis and pulmonary emphysema, and bilateral conjunctivitis.

Laboratory results: PCV 17 with ++ polychromasia WBC - 9500, 69% heterophils, 13% lymphocytes, 15% monocytes, 2% eosinophils 1% basophils
K+ 8.5 mmol/L, Uric Acid >25mg/dL, Bile Acids= 35 umol/L, Ca+ 20 mg/dL

Microscopic Description:
A section of terminal colon is presented. The long folds are an anatomic normal. Large segments of mucosa, especially the length of the folds, are covered by necrotic mucosa, cell debris and heterophils and macrophages, and saccules of the colon are distended by mucus with a mild, necrotic, pleocellular detritis and numerous bacteria. Viable

mucosa is disorganized and not the normal, simple, columnar epithelium. Colonic epithelium is piling up. Cells have large, hypochromic nuclei with prominent nucleoli and with somewhat granular, poorly defined eosinophilic cytoplasm. Small numbers of granulocytes exocytose. The submucosa has distended vessels (congestion) with some extravasated erythrocytes, and tissues are rarified (edema) with a mild to moderate granulocytic, histiocytic and lymphocytic infiltrate. The muscularis is intact, and coelomitis is not noted.

In the necrotic cell debris and in the viable epithelium are many, 20-35u amoeba trophozoites with usually a single endosome and granular or globular cytoplasm (*Entamoeba* sp.). Additionally, many of the epithelial nuclei have variable numbers (1 to 8) of eosinophilic meronts. Pairs and clusters of these meronts, and clusters like merozoites of these coccidian are noted. Rare megaschizonts may be seen.

Contributor's Morphologic Diagnoses: Necrogranulocytic/heterophilic, hyperplastic and mucinous colitis with amoeba and intranuclear coccidian.

Contributor's Comment: This is apparently a common presentation in land tortoises, based on cases published. [3] The animal was euthanized; however, she had a severe enterocolitis. The severity of the GI
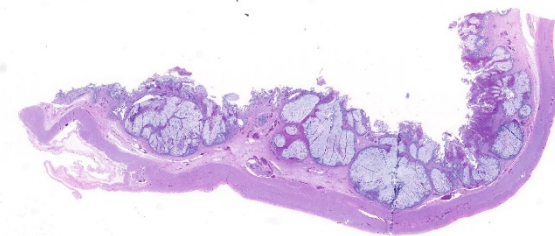
Colon, leopard tortoise. There is multifocal necrosis of the colonic mucosa which is replaced by eosinophilic coagulum. (HE, 7X)

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lesion and the two associated pathogens were the reasons for the choice of our tissue selection. The coexistence of the presumed *Entamoeba* and intranuclear coccidian is not unique to our case, because it is reported.

Because no bacterial pathogens or their toxins (*Salmonella, Clostridium, Citrobacter etc.*,) were recovered from this tortoise, the *Entamoeba* was considered a significant pathogen. [2,6,9,13] *Entamoeba invadens* has been isolated from a leopard tortoise, but it should be remembered that this organism is considered a commensal to most species of chelodons. Epizootics associated with large numbers of amoebae in reptiles are reported[6], but many are not tested with molecular procedures. It has been reported that the morphology of amoeba especially the morphology of the trophozoite in tissue is not adequate to speciate this protozoa. [2,13] Diagnosticians must start testing, culturing

and collecting fecal cysts and typing these organisms to establish *bona fide* pathogenic strains. It is interesting that seven species of tetranucleated-cyst-producing *Entamoeba* (*E. invadens* has 2-4 nuclei in cysts) have been associated with chelonian hosts.

In this particular case, deep ulcers were not seen in the colon, and amoeba were not associated with hepatic lesions; however, *Entamoeba* was presumed to be a significant pathogen in the colon, even though it did not burrow deeply because such large numbers of organisms are visible in viable colon as well as in the necrotic debris. One report of entamoebiasis in a leopard tortoise was associated with a myositis (not seen in our case).[10] A similar, verminous colonic lesion has been reported associated with *Proatractis sp*., but no embedded parasites were seen in our case.[10]
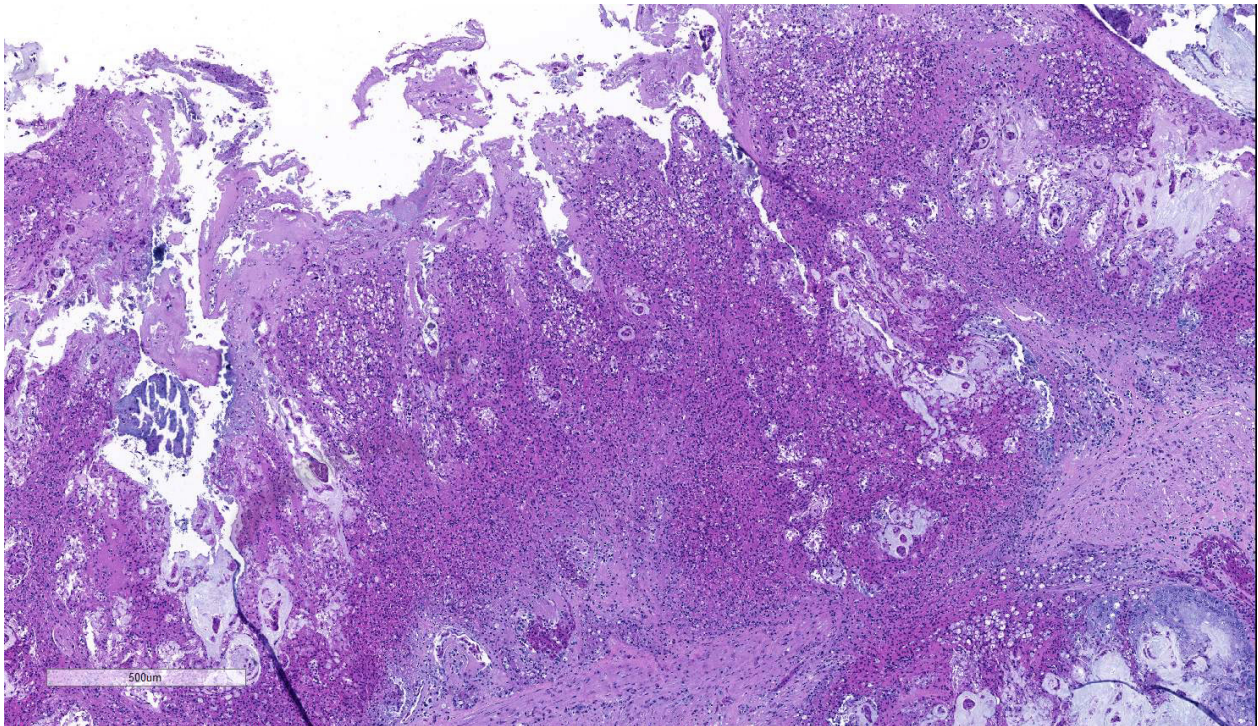
Colon, leopard tortoise. Higher magnification of necrotic areas of the colonic mucosa.   (HE, 47X) (Photo courtesy of: Department of Veterinary Pathobiology, College of Vet Med and Biomedical Sciences, Texas A&M University, College Station, TX USA 7784

Intranuclear coccidia are reported in chelodons.[1,3,5,7,8,12] The identity of the intranuclear coccidian is not known, but one report suggests it is a member of the Sarcocystidae while another suggests their case organism may be closely related to an *Eimeria*.[1,5,12] Multisystemic infections similar to our case also are reported in leopard tortoises. The proliferative lesion of the colon is presumed to be due to the coccidian, perhaps similar to the proliferative lesion seen in sheep and goats with eimeriosis. Coccidia affected several systems in this individual. The colonic intranuclear forms are varied.[3,5,7] The renal coccidial infection was striking in our case and no doubt was responsible for the renal compromise. The conjunctivitis was associated with coccidian as well: however, we did not culture for Mycoplasma (especially *M. agassizii*), a conjunctivitis-associated pathogen in tortoises.[7] The liver was lipidotic, and no cytoplasmic coccidial forms were seen in hepatocytes as reported in one published case, but intranuclear coccidians were occasionally seen in biliary epithelium. It is interesting to speculate that some nervous signs may be seen with coccidiosis as seen in cattle!
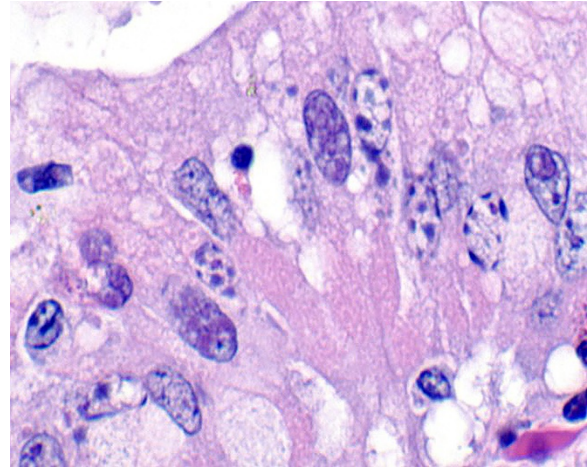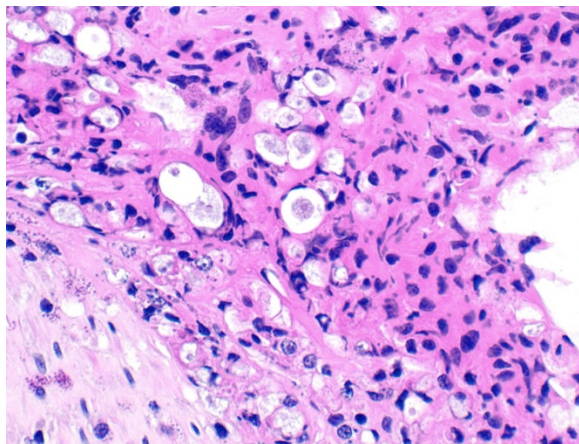

Colon, leopard tortoise. Mucosal epithelium contains variable numbers of apicomplexan zoites within their nuclei. (HE, 400X) (Photo courtesy of: Department of Veterinary Pathobiology, College of Vet Med and Biomedical Sciences, Texas A&M University, , College Station, TX USA 77843-4467)

Colon, leopard tortoise. Trophozoites of Entamoeba invadens are scattered throughout the debris. (HE, 400X) (Photo courtesy of: Department of Veterinary Pathobiology, College of Vet Med and Biomedical Sciences, Texas A&M University, College Station, TX USA 77843-4467)

We did not test for either Type I or II chelodon herpesvirus, perhaps an oversight, but we were overwhelmed by the enterocolitis. Any herpesviral inclusions would be difficult to distinguish from intranuclear coccidian forms without EM or immunotesting. A causal relationship between chelodon herpesviruses and any of the herpesvirus related conditions is sometimes tenuous. The adenoviral nuclear inclusions, however, would seem to be distinguishable however.[12]
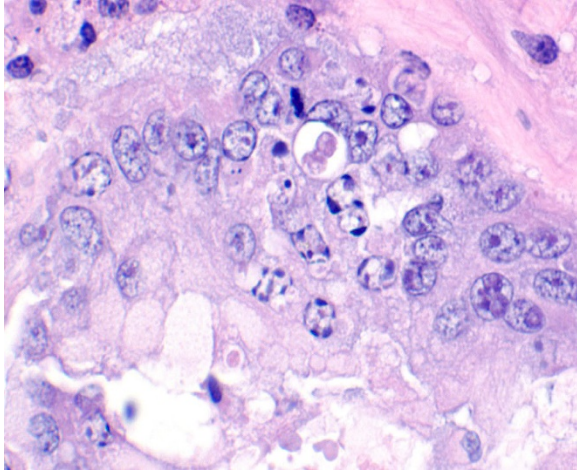
Contributing Institution:
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College of Vet Med and Biomedical Sciences
Texas A&M University
College Station, TX USA 77843-4467

Colon, Mucosal epithelium contains eosinophilic intranuclear zoites and a single oocyst (arrow). (Photo courtesy of: Department of Veterinary Pathobiology, College of Vet Med and Biomedical Sciences Texas A&M University, College Station, TX USA 77843-4467)

JPC Diagnosis: Colon: Colitis, necrotizing, multifocal to coalescing, subacute, severe with numerous amebic trophozoites,, and intranuclear apicomplexan zoites and schizonts.

JPC Comment: *Entamoeba invadens* is a common parasite of reptiles which occasionally affects turtles. Within collections, freshwater turtles are considered to be a source of infection for other reptile species; snakes may be especially hard hit. The genus *Entamoeba* infects many classes of vertebrates, but only a few species have been proven to cause disease. *Entamoeba suis* has been identified in swine with hemorrhagic colitis. Several species of *Entamoeba* infect man and non-human primates: *Entamoeba histolytica* the cause of "amebic dysentery" and rare cases of extraintestinal disease, as well as non-pathogenic species *E. dispar and E. moshkovii*.[12] Intestinal colonization occurs with encysted parasites and infection can be spread by asymptomatic carrier by oral-fecal routes. Excystation of the organism results in production of trophozoites which invade the intestinal wall leading to ulcer formation and diarrhea.[12] Trophozoites are capable of penetrating the intestinal wall and can lead to more severe complications including liver abscesses (the most common) and, in rare cases, can spread to the brain and/or lungs, which is often fatal.[12] Recent *Entamoeba* cases in the Wednesday Slide Conference include a case of *E. invadens* in the intestine of a green anaconda (WSC 2013-2014, Conf. 21, Case 4; and *E. histolytica* in the liver of a guereza monkey – WSC 2010-2011, Conference 6, Case 1.)

Intranuclear coccidia have been sporadically reported since the 1990s and found in a wide range of chelonians to include leopard, Sulawesi, and giant tortoises, among others, as well as Eastern box turtles.[10] They are considered systemic infections and widespread in chelonian collections. Clinical presentation of this disease is variable and includes mucoid nasal and/or ocular discharge (seen in this case), and it may exist as a coinfection with amebiasis (seen here), mycoplasmosis, or bacterial septicemia. Gross lesions may include generalized subcutaneous edema, coelomic effusion, necrotizing enteritis, systemic petechiation, and skin ulcers. Intranuclear coccidia may be present in a wide range of cells, including enterocytes and renal tubular epithelium as seen in this case, hepatocytes, biliary epithelium, pancreatic ductular epithelium and acinar cells, urinary bladder epithelium, nasal mucosa, and tracheal epithelium and pneumocytes.[10] Recently, oro-fecal transmission has been established as the method of transmission between individuals.[4]

While most commonly identified in chelonians, intranuclear coccidia may also been seen in other species. *Cyclospora caryolytica* and *Cyclospora talpae* are present within the small intestine and liver, respectively, of moles, and other species of
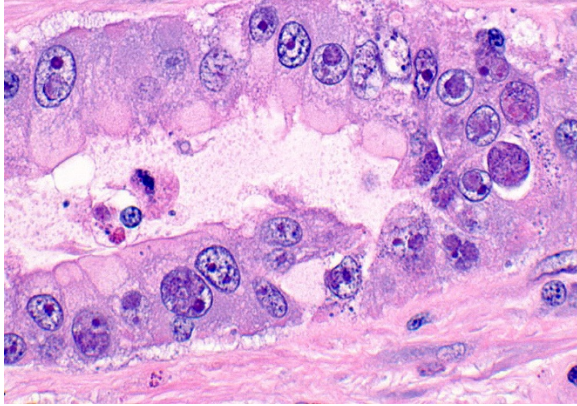
Kidney, leopard tortoise. Tubular epithelium contains eosinophilic intranuclear zoites as well. (Photo courtesy of: Department of Veterinary Pathobiology, College of Vet Med and Biomedical Sciences, Texas A&M University, College Station, TX USA 77843-4467)

Cyclospora have been identified as causing watery diarrhea in Japanese Black calves. [14] *Eimeria alabamensis* and *E. subspherica* have also been identified in diarrheal disease of calves, also including Japanese black calves (*E. subspherica*.[14] *Eimeria hermani* and *E. stigmosa* have been observed in the enterocytes of geese.[14]

The moderator mentioned that the cause of pyramiding is actually due to low humidity, rather than nutritional causes (although a concurrent nutritional cause cannot be totally ruled out.)

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CASE III: 15-41009 (JPC 4084545).

Signalment: Adult, intact male tiger (*Panthera tigris*)

History: The animal presented for necropsy examination following euthanasia after a 3-week history of progressive pneumonia that was unresponsive to antibiotic administration.

Gross Pathology: The lungs were mottled pink to red and rubbery to firm on palpation. The right middle lung lobe was more meaty and consolidated than the other lobes. There were also multiple pale tan, round, variably sized up to 2 cm in diameter nodules interspersed throughout the pulmonary parenchyma. The bronchi within the cranioventral lungs were often filled with frothy and purulent material.

Laboratory results: Virology: The lungs were positive with fluorescent antibody testing for Canine Distemper Virus. Virus isolation was unsuccessful.
Bacteriology:
PCR on lung tissue for Mycoplasma sp. was negative.
Aerobic Culture and identification using MALDI-TOF -

| Specimen	Isolate	Level
Lung	Acinetobacter sp.	few
Result Comment:		
Identified by MALDI-TOF as A. haemolyticus with a score of 2.331.		
Lung	Escherichia coli	v rare
Lung	Ochrobactrum	v few
Lung	Pseudomonas pseudoalcaligenes few	
Lung	Gram-negative rod	v few
Result Comment:		
Identified as Rhizobium radiobacter by MALDI-TOF with a score of 2.308.		
Lung	Alcaligenes faecalis	v rare

General Test Comment:
MALDI-TOF Score:
Range Description
2.300…3.000 Highly probable species identification
2.000…2.299 Secure genus identification, probable species identification
1.700…1.999 Probable genus identification
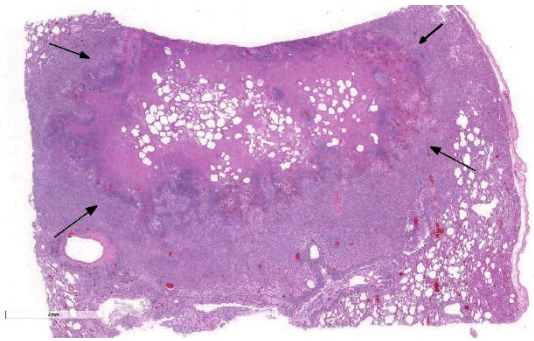
Lung, tiger: There is a focally extensive area of infarction (arrows). Much of the rest of the section is hypercellular. (HE, 5X)

Microscopic Description:

Lung: Approximately 80% of the section is affected by a profound inflammatory process. Alveolar spaces are frequently filled with many viable and degenerate neutrophils mixed with foamy macrophages, abundant eosinophilic, fibrillar material (fibrin), moderate cellular debris, and mild hemorrhage. The alveolar septa are diffusely thickened up to 5 times their normal diameter by moderate numbers of similar inflammatory cells, marked type II pneumocyte hyperplasia, and moderate smooth muscle hypertrophy. Pneumocytes often form multi-nucleated syncytial cells that contain up to 7 nuclei. Many pneumocytes and syncytial cells contain one to several variably sized and shaped, eosinophilic, glassy, intracytoplasmic and intranuclear inclusion bodies. There is multifocal, extensive necrosis with abundant pyknotic,

karyorrhectic, and karyolytic cellular debris, and alveoli, bronchioles and bronchi in these areas are frequently filled with innumerable degenerate neutrophils mixed with fewer macrophages and eosinophils. Many macrophages are enlarged up to 70 µm in diameter and contain an intracytoplasmic, 20 x 15 µm protozoal cysts that are filled with small, 1-2 µm bradyzoites, as well as non-encysted and similarly sized tachyzoites. Cysts and tachyzoites are also present free within the extracellular space.

Contributor's Morphologic Diagnoses:

Lung: Diffuse, marked, fibrinonecrotic, suppurative, histiocytic, bronchointerstitial pneumonia with intracytoplasmic and intranuclear epithelial inclusions (Canine Distemper Virus) and protozoal cysts and free zoites (*Toxoplasma gondii*).
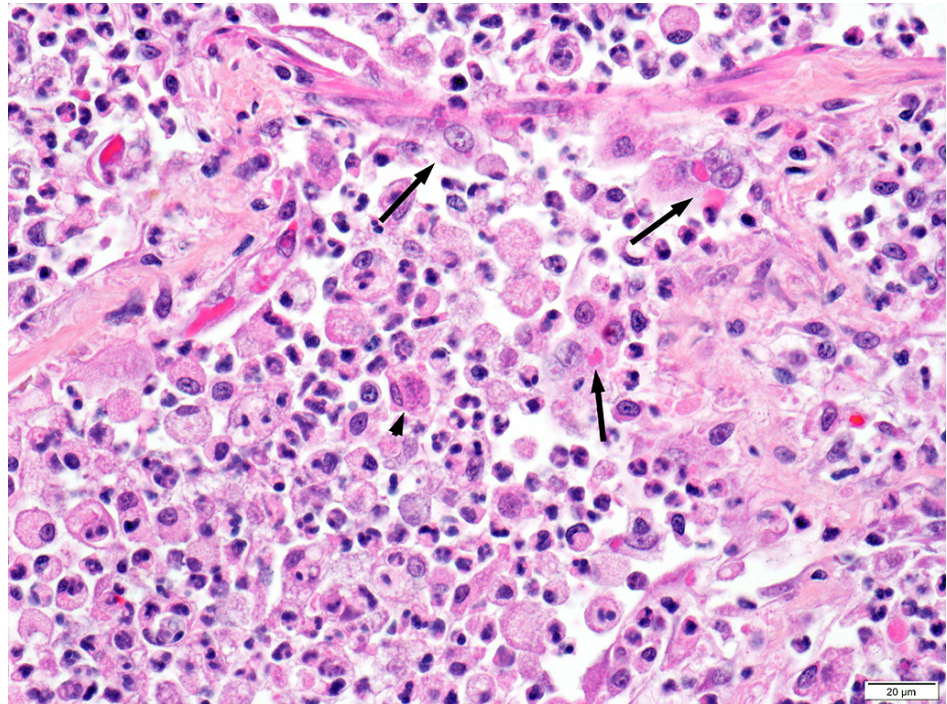
Lung, tiger: Inflammatory cells fill alveolar spaces and expand alveolar septa. Viral inclusions are presents within pneumocytes and syncytial cells (arrows) and a Toxoplasma cyst is within a macrophage (arrowhead). (HE, 400X)
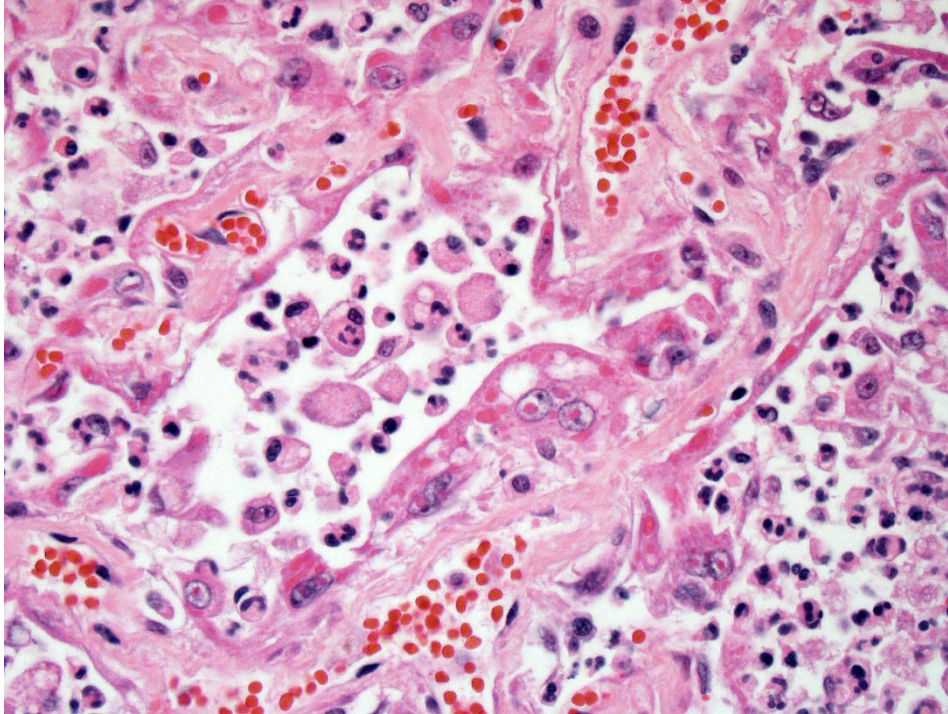
Lung, tiger: Type II pneumocytes contain numerous intracytoplasmic and intranuclear inclusions and often have multiple nuclei (viral syncytia). (HE, 400X) (Photo courtesy of: University of Illinois College of Veterinary Medicine, Department of Pathobiology, 2522 Veterinary Medicine Basic Science Building, 2001 S. Lincoln Ave., Urbana, IL, 61802, http://vetmed.illinois.edu/research/departments/pathobiology/)

Contributor's Comment: Canine distemper virus (CDV) inclusions were also identified within the glandular epithelium of the gastric mucosa in this case. There were no identified abnormalities within the nervous system. *Toxoplasma gondii* organisms were also found within a tracheobronchial lymph node. Additional findings in this case included multiple mast cell tumors within the liver and spleen. This animal was part of a larger outbreak of CDV in a group of big cats.

CDV is in the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Morbillivirus*. It is a 100–700 nm, irregularly shaped virus composed of an outer lipoprotein envelope, and inner matrix, and a nucleocapsid that contains single-stranded negative-sense RNA Infection occurs by inhaling aerosols or close contact with infected animals. The virus enters via the epithelium and infects upper respiratory tract macrophages which transport it to local lymphoid tissues where the virus replicates further, and spread continues unless adequate cell-mediated and humoral responses are mounted.[7,11] Clinical signs in CDV infection most often include the respiratory, nervous, and gastrointestinal systems. Many other tissues may be affected as well. Key histologic changes in CDV infection are eosinophilic, homogenous or glassy, variably sized intranuclear and intracytoplasmic inclusion bodies, which are most easily identified in the nervous system and epithelial tissues. In the lungs, commonly observed changes include bronchointerstitial pneumonia accompanied by viral inclusions within airway epithelium, epithelial syncytial cell formation, and type II pneumocyte hyperplasia.[7]

The host range of CDV is wide and continuing to grow. A recent retrospective review[5] compiled all reported natural and experimental CDV infections. Susceptible animals include those from *Carnivora* (*Canidae, Felidae, Mustelidae, Procyonidae, Hyaenidae, Ursidae, Phocidae, Viverridae, Ailuridae, Mephitidae, Odobenidae, and Otariidae*), *Rodentia* (*Muridae, Cricetidae,*
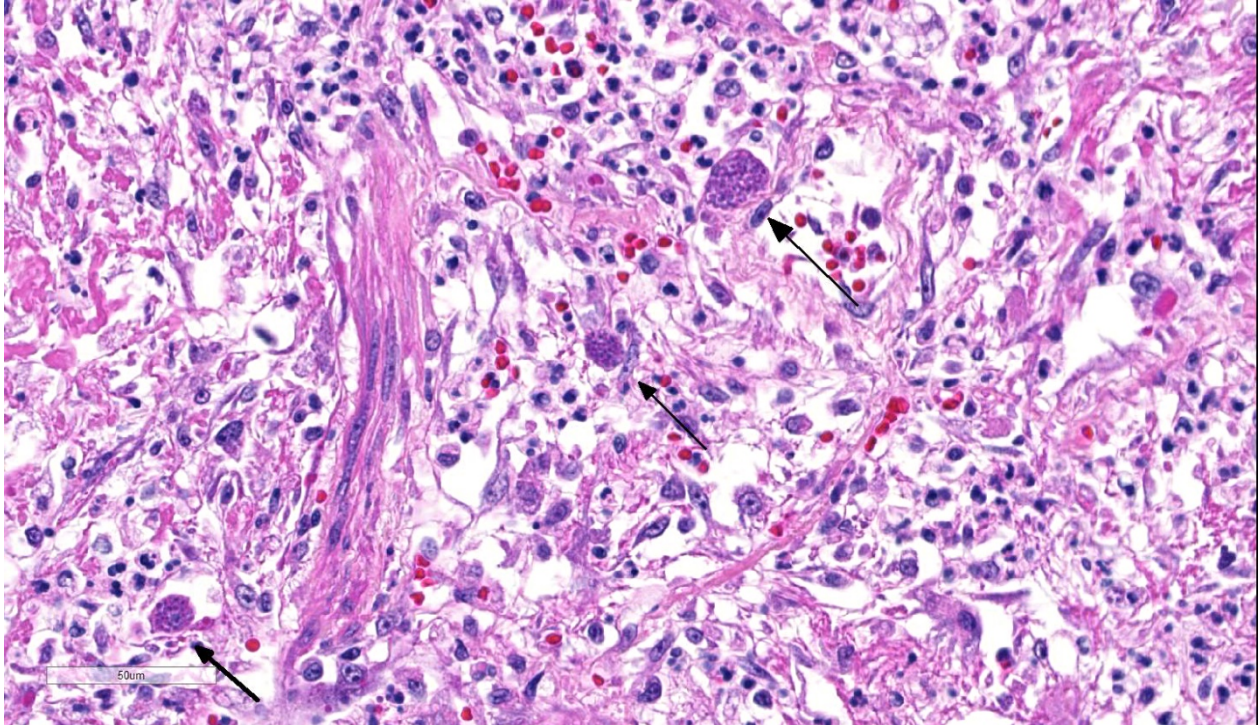
Lung, tiger: Numerous Toxoplasma cysts are present within areas of necrosis and/or fibrosis (arrows). (HE, 400X)

Sciuridae, and *Caviidae*), Primates (*Cercopithecidae* and *Cebidae*), *Artiodactyla* (*Suidae*, *Tayassuidae*, and *Cervidae*), and *Proboscidea* (*Elephantidae*) are susceptible to CDV.[5]

Toxoplasma gondii is a heteroxenous apicomplexan protozoan parasite that is capable of infecting virtually all homeothermic animals. It is an obligate intracellular parasite. Felids serve as definitive hosts, while other animals are intermediate hosts. Many if not all cells and tissues are susceptible to infection. Infection of non-intestinal tissues is achieved by initial ingestion of oocysts, excysting of sporozoites followed by their penetration of small intestinal mucosal and lamina propria cells, and replication within these small intestinal cells. With continued replication and formation of tachyzoites, cells eventually rupture, and tachyzoites infect new cells and are capable of spreading to distant tissues via blood or lymph.[4,6] Necrosis predominates in

gross lesions observed and typically presents as variably sized gray foci within any tissue. Within the lung, microscopic changes closely follow that of interstitial pneumonia. Alveolar septa are initially thickened by mononuclear cells with fewer granulocytes, followed closely by type II pneumocyte hyperplasia. Alveolar lumina are filled with macrophages and fibrin. Necrosis is abundant with continued parasitic replication. Intra- or extracellular cysts and zoites within affected areas are frequently observed and are highly suggestive of toxoplasma infection.[4,6]

Infection is believed to be widespread among humans, up to 80% in some parts of the world. (7) Seroprevalence is high in several studies involving zoo and wild animals, up to 90% in wild animals (foxes), and 52.4%, 64.5%, and 81.4% in zoo felids.[1,3,9,10] *Toxoplasma gondii* has high zoonotic potential, and though high seroprevalence with little clinical disease indicates that most
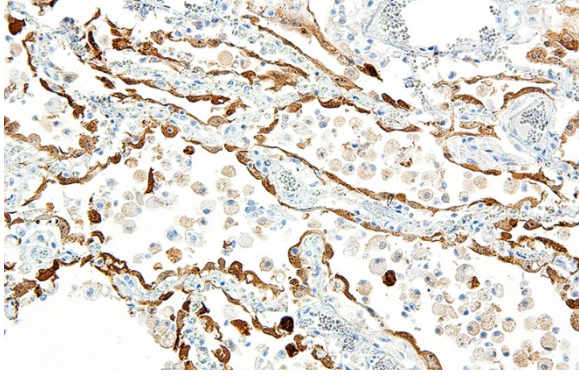
Lung, tiger. Pneumocytes and occasional macrophages have strong cytoplasmic reactivity to canine morbillivirus immunohistochemistry. (anti-CDV, 200X) (Photo courtesy of : University of Illinois College of Veterinary Medicine, Department of Pathobiology, 2522 Veterinary Medicine Basic Science Building, 2001 S. Lincoln Ave., Urbana, IL, 61802, http://vetmed.illinois.edu/research/departments/pathobiology/)

infections are asymptomatic, infection in immunocompromised individuals can cause severe illness and often death. With the current case, the tiger was undoubtedly immunocompromised from concurrent CDV infection.

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JPC Diagnosis: Lung: Pneumonia, bronchointerstitial, , necrotizing and fibrinosuppurative diffuse, severe, with numerous intraepithelial intracytoplasmic and intranuclear viral inclusions viral syncytia and intraepithelial and intrahistiocytic apicomplexan zoites.

JPC Comment: Transpecies infection with canine morbillivirus is not a new phenomenon. Canine morbillivirus infection (CDV) in non-canine host as first reported in silver jackals in 1937 in South Africa; the first cross-species infection in the U.S. (the country with the largest number of confirmed reports occurred in a badger in Colorado in 1942.[5] Inter-Order infection was first accomplished experimentally in hamsters in the early 1960s (but required cerebral inoculation.)[5] Natural intra-order infection occurred in the late 1970s and 1980s in primates. While transspecies infection by CDV (and high mortality) is primarily in carnivore species (prompting at least one call to rename it "Carnivore Distemper Virus",[12] herbivorous species such as collared peccaries and non-human primates are documented as well.[2] Outbreaks of morbillivirus disease in seals may result from either CDV or closely related phocine distemper virus. Dolphins, harbor porpoises and pilot whales all have genetically different morbillivirus which cause similar disease (and have been transmitted to Mediterranean monk seals, but interestingly, their morbillivirus appears more closely related to the morbillivirus causing *pestis du petits* in ruminants and that which caused rinderpest.[2]

Rarely associated with non-human primates, CDV infection has occurred in a number of species of macaques in Japan and China since the first case occurred in Japanese macaques in 1989.[8] CDV was responsible for up to 4000 rhesus fatalities in a breeding program in China, in which animals displayed measles-like symptoms with respiratory distress, rashes over the entire body, reddening and swelling of the footpads, conjunctivitis, and a thick nasal discharge. Interstitial fibrosis was a predominant finding at autopsy. Following sequencing of the viral genome, it showed 97% homology with other
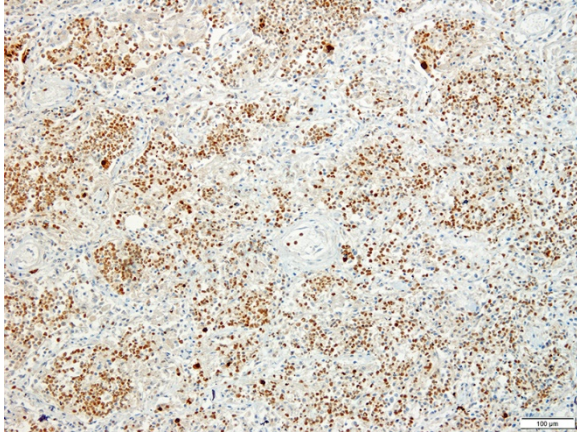
Lung, tiger. Toxoplasma cysts and macrophages demonstrate strong cytoplasmic immunoreactivity to Toxoplasma immunohistochemistry. (anti -T. gondii, 100X). (Photo courtesy of : University of Illinois College of Veterinary Medicine, Department of Pathobiology, 2522 Veterinary Medicine Basic Science Building, 2001 S. Lincoln Ave., Urbana, IL, 61802, http://vetmed.illinois.edu/research/departments/pathobiology/)

CDV isolates from China. A source for this and related outbreaks was not identified.[8]

Molecular evolutionary analysis of CDV has suggested that mutations in the signaling lymphocyte activation molecule (SLAM, CD150 - the common receptor used by morbillivirus to gain entry into the host cell - and the CDV hemagglutinin (HA) protein that binds the SLAM molecule are important in cross-species infection as well as species specificity. [2,12] An area of particular concern in the future would be the potential for CDV strains that infect primates, which already are able to use the human nectin-4 receptor for cellular entry, to mutate in the region of the viral H protein and become infective for humans.[2]

The moderator reviewed the history and epidemiology of morbillivirus infection in since the establishment of the veterinary school in Lyon, France in 1762 (in order to combat periodic outbreaks of rinderpest in Europe.)

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CASE IV: M17-69 (JPC 4102647).

Signalment: Juvenile (age unknown), female, American paddlefish (*Polyodon spathula)*

History: The fish was found dead in a stable aquarium system.

Gross Pathology: Multiple fixed tissues were received for histopathology as a mail-in necropsy. The fish was reported to be in fair to poor physical condition. Multifocal, widespread, 1-2 mm, white to tan nodules were observed on serosal and cut surfaces of the stomach and pyloric ceca during tissue trimming.

Laboratory results: N/A

Microscopic Description:
Stomach: The submucosa and muscularis are widely expanded by severe fibrosis and inflammatory infiltrates surrounding large

Stomach, paddlefish. Granulomas centered on larval nematodes transmurally throughout the wall. (HE, 4X)

numbers of larval nematodes. Features of the pseudocoelomate parasites include a smooth cuticle, coelomyarian/polymyarian musculature, lateral cords with eosinophilic gland cells, triradiate glandular esophagus, cecum, and large intestine lined by tall uninucleate columnar cells with a brush border. Worms are coiled within cavitated lesions partially filled by loosely associated mixtures of degenerate cells, necrotic cellular debris, erythrocytes and mixed inflammatory cells. More organized, unevenly thick mantles, composed of variable mixtures of eosinophilic granulocytes, macrophages, lymphocytes, plasma cells and fibroblasts gradually dissipate into the adjacent gastric wall. The lamina contains sparse, often short glands, with reduced cytoplasm and cytoplasmic granules, widely separated by abundant dense collagenous tissue.

Contributor's Morphologic Diagnoses: Stomach: Gastritis, mural, widespread, chronic, severe, with glandular atrophy and loss, and larval nematodes

Contributor's Comment: The American paddlefish is a large, filter-feeding, cartilaginous, freshwater fish that is closely
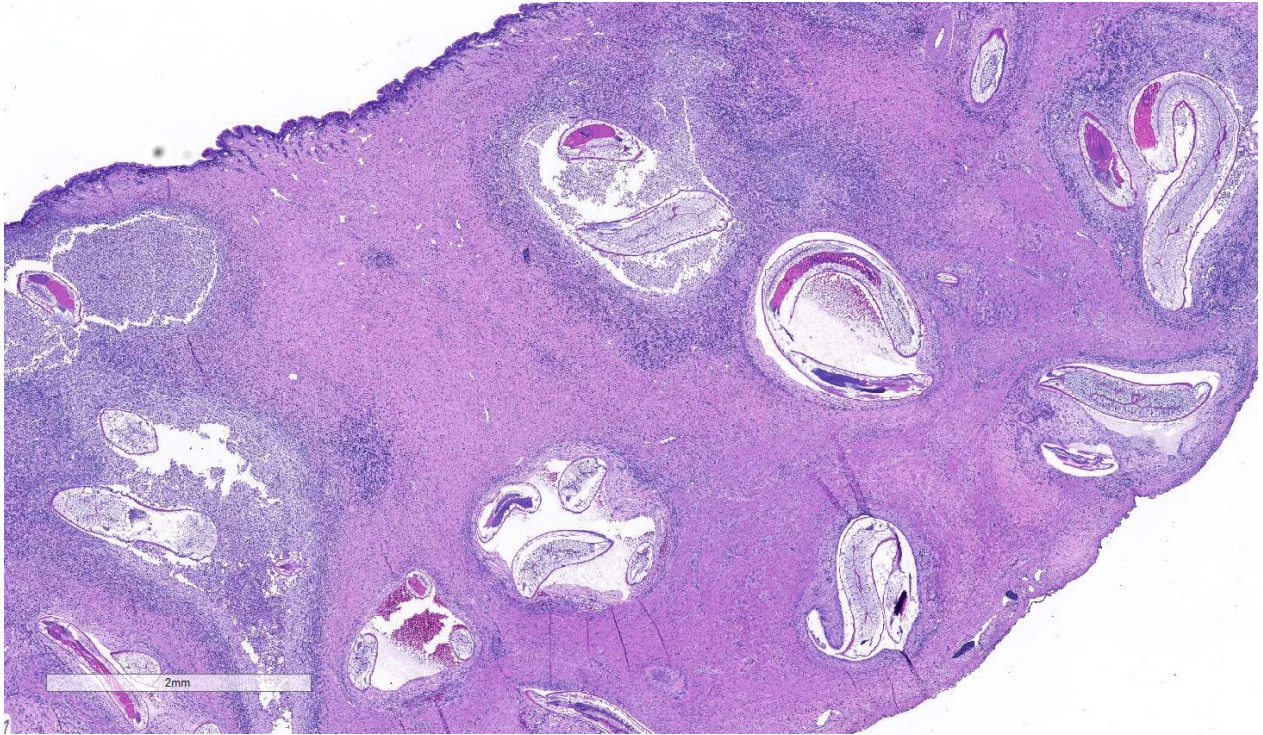
Stomach, paddlefish. Higher magnification of the intestinal wall with numerous 400um diameter larval nematodes contained within granulomas. (HE, 32X)

related to the sturgeons. Paddlefish roe, like that of several sturgeon species, is highly valued as caviar and supports a growing aquaculture industry.[4] Microscopic examination revealed severe inflammatory changes and fibrosis, with glandular atrophy and loss in the stomach and cecal walls in association with large numbers of larval nematodes. Features of the worms and the associated histologic changes are compatible with descriptions of the anasakid nematode *Hysterothylacium dollfusi*, a major parasite of and impediment to the pond-culture of paddlefish.[1,5] Although gastric ulceration was not present in the sections evaluated, the severe changes and glandular loss suggest maldigestion and malabsorption, consistent with the poor condition of this fish. Additional findings included severe hepatocellular and pancreatic acinar cell atrophy suggesting prolonged anorexia and catabolism of hepatic lipid stores. The Anisakidae are a family of intestinal nematodes, including the well-known genera *Anisakis*, *Contracaecum* and *Pseudoterranova*. Representative *Anisakis* spp., most notably *A. simplex*, and *Pseudoterranova* spp. have the potential to cause zoonotic infections in humans (anisakidosis) when larvae are ingested in raw or insufficiently cooked fish. Anisakidosis caused by *Contracaecum* and *Hysterothylacium* spp. are rare. The life cycles of the genera are complex, involving an invertebrate first intermediate host, fish or cephalopod second intermediate host and often a bird or marine mammal final host.[2,3]

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JPC Diagnosis: Stomach, muscularis and serosa: Granulomas, numerous with larval
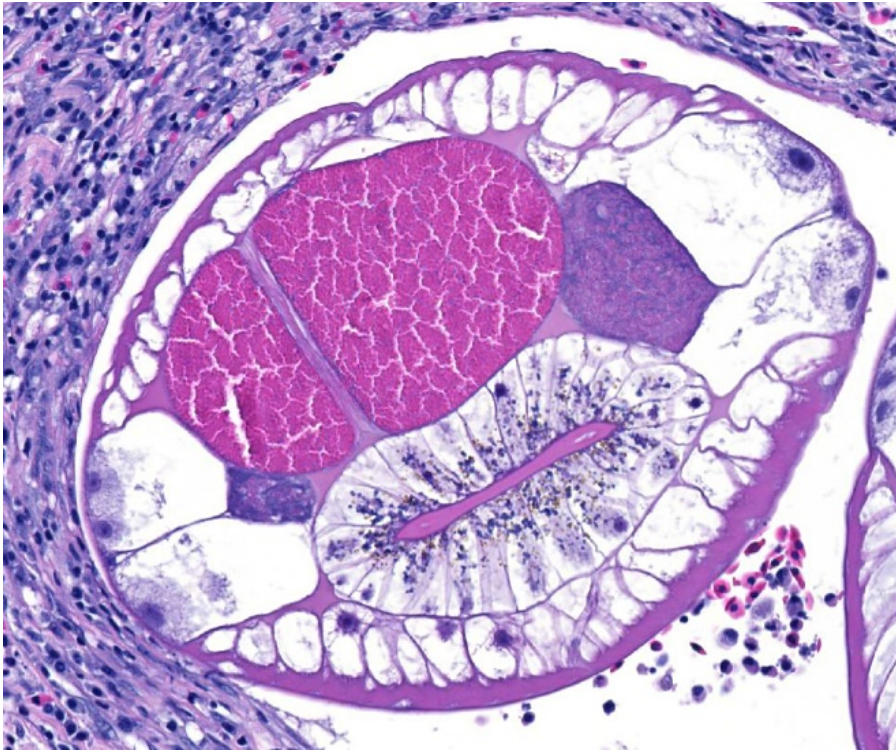
Stomach paddlefish. Larval ascarids have a thin cuticle, high polymyarian-coelomyarian musculature, prominent lateral cords with excretory cells, developing gonads, and a GI tract with tall uninucleate columnar epithelium and a developing gonad (HE, 273X)

ascarids and mild chronic granulocytic and lymphoplasmacytic gastritis.

JPC Comment: *Anisakis* sp. and *Pseudoterranova sp.* the causative agents of anisakidosis in humans, utilize marine fish as paratenic hosts and whales and dolphins and seals and sea lions, respectively, as definitive hosts. Humans, which may develop abdominal pain, diarrhea and nausea (as well as strong allergic reactions) as accidental nonpermissive hosts. The infection of humans by species of *Anisakis* is called "anasakiasis", while "anisakidosis" refers to infection by any member of the Anisakidae. [6]

Parasitized humans may experience abdominal pain within a few hours to a few days following ingestion of infected raw fish. Infection may occur in the stomach (where worms may be removed by gastroscopy, or the intestine (which often requires surgery and may result in obstruction as a result of severe inflammation (which may expand the intestinal wall 3-5 times. Rare cases of "ectopic anisakiasis" may result from larval penetration within the pharynx, tongue, lung, peritoneal cavity, or pancreas. Another form of anasakidosis is gastroallergic anasikidosis, which may not present with gastrointestinal symptoms, but instead with various degrees of urticarial, angioedema, and anaphylaxis. In fact, the vomiting and diarrhea associated with allergic reactions, may actually expel the larva from the body[6]. Interestingly, gastric allergic anisakiasis are relatively common in certain countries, including Spain and Italy, has been reported in Japan and Korea, but is rare in other parts of the world – possibly due to awareness of the condition in these parts of the world. It is not clear whether it takes live larvae or antigens from dead larvae to set off a gastroallergic reaction, as patients with previous gastroallergic anisakiasis will not react to exposure to dead larvae or antigens, while fish processing works may develop *Anisakis*-induced asthma, rhinoconjunctivitis, or dermatitis.[6]

In humans, larvae that penetrate the gastric wall, but usually die within a few days in humans. The larvae will break down in eight weeks, with eosinophils being a large component of the inflammatory lesion. Dead

Anisakis larvae may incite granuloma formation within tissues and may be associated with systemic neutrophilia or eosinophilia.[6] The presence of inflammatory nodules may be mistaken for neoplasia, highlighting the importance of taking a good history in cases of acute onset of epigastric pain and intestinal symptoms.

In addition to reviewing anisakiasis in animals, the moderator reviewed additional large zoonotic heminthic disease, to include dracunculiasis and diphyllobothriasisis.

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