Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #11 30 November 2022

CASE I:

Signalment:
Adult female giant Pacific octopus (*Enteroctopus dofleini*)

History:
Octopus arrived to the National Aquarium in Baltimore bright, alert, and responsive, but missing multiple distal limb pieces, suspected to be traumatic in nature. Two weeks after capture, this octopus was noted to be hyporexic. Fecal examination was concerning for cystic structures, possibly *Aggregata sp*. Octopus was noted to be dark in color and posturing strangely the next morning, reportedly standing on its limbs. The animal was found deceased after lunch; lack of heartbeat was confirmed via Doppler and ultrasound. Prior to death, water temperature was noted to be within normal limits and dissolved oxygen content at 99%.

Gross Pathology:
The submitting institution performed the gross examination which identified partial traumatic amputation of the distal arms of R1-3, L2, and L4, as well as marked gill pallor. Hemolymph drawn after death was clear when drawn, but turned deep blue (aerated) after 1 minute in the collection tube. The kidneys were also said to contain multiple pinpoint white spots of unknown significance.

Laboratory Results:
Below are values from the aforementioned post-mortem sample; published reference intervals (RI) for hemolymph in octopus are not available.
Hemocyte count: 2.255k/uL (empirically low)
Copper: 174.925 mcg/dL (likely appropriate, RI unknown)
Chemistry: Glu 20 mg/dL (empirically low), *Na 376.2 mmol/L (likely appropriate, RI unknown), K 12.6 mmol/L (empirically high), Cl 402 mmol/L (empirically high)*, TP 11.4 g/dL (empirically very high)

Microscopic Description:
Gill: Moderate numbers of macrogametes are embedded in the connective tissue of the gill lamellae. These round to ovoid cystic structures, with a central nucleus and a large, darkly staining nucleolus, measure up to 100

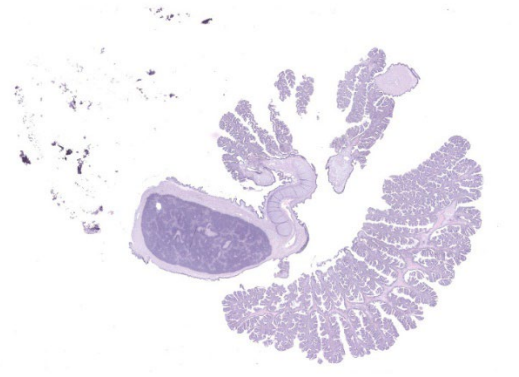

Figure 1-1. Gill lamellae, octopus. A cross section of the gill is submitted for examination. There is no abnormality at subgross magnification. The branchial gland is at left. (HE, 5X)

μm in diameter and frequently invade the gill epithelium. Cysts contain abundant finely granular or vacuolated basophilic cytoplasm. Hemocytic infiltration of the affected gill lamellae is variable, with some areas of severe hemocytic inflammation noted in the interstitium of the gill lamellae. Hypertrophy of infected epithelial cells is quite prominent in some regions. The centralized branchial gland is unaffected. In some sections, low numbers of flagellate protozoa are noted adherent to the apical surface of gill epithelial cells, consistent with *Ichthyobodo sp.*

Contributor's Morphologic Diagnoses:
Gill lamina: coccidiosis, multifocal, moderate with intralesional macrogametes.

Contributor's Comment:
The eimeriorin coccidian protozoal organisms of the genus *Aggregata* and the greater phylum Apicomplexa are frequently found to infect the digestive tract of a diverse number of cephalopods. These parasites can cause damage to the host tissue including direct mechanical injury, as well as indirect enzymatic and innate immune effects.[2] This leaves cephalopods under considerable stress, most commonly those housed in fishery or aquaculture situations, vulnerable to secondary infections. Additionally, it has been reported

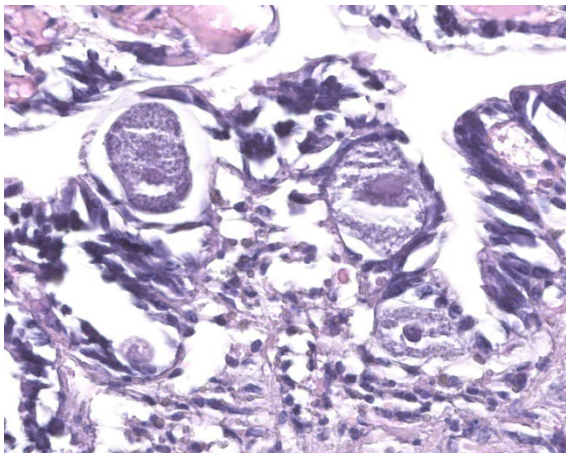

Figure 1-2. Gill lamellae, octopus. Apicomplexan gametocytes with granulated cytoplasm and a single homogenous nucleus are embedded within the hyperplastic epithelium. (HE, 574X)

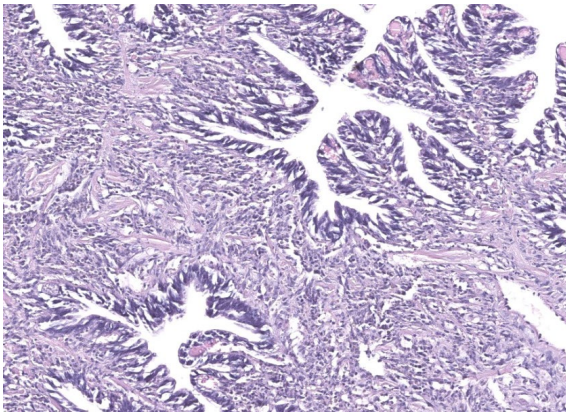

Figure 1-3. Gill lamellae, octopus. The connective tissue of the gill lamella is infiltrated by large numbers of hemocytes. (HE,144X)

that the enzymatic disturbances that occur in octopods result in malabsorption, producing a gradual decline in protein levels, emaciation, and even death.[3] In the past 25 years, *Aggregata spp.* organisms have been considered the most important disease-producing infectious agent in both wild and cultured populations of the common octopus (*Octopus vulgaris*), a major protein source in many fish-eating countries.[4] Infections have previously been described in the intestinal tracts of giant Pacific octopuses (*Enteroctopus dofleini*) from this colony as the result of a newly identified species, *Aggregata dobelli*.[8]

Aggregata spp. coccidia have heteroxenous development with the asexual stages (merozoites, meronts) invading the intestinal epithelial cells of crustaceans, the intermediate host, until subsequent ingestion by cephalopods.[2] These merogonial stages can then infect the digestive tract of the definitive host, as well as the epithelial cells of other tissues, such as the gill lamellae. To gain access to these extraintestinal sites of infection, the invasive oocysts are able to migrate through tissues, causing hypertrophy of invaded cells, hemocytic infiltration, and, consequently, phagocytosis by these hemocytes.[5] Oocyst and macrogamete stages are frequently observed embedded in the connective tissue and surrounded by a thin cyst wall or a multi

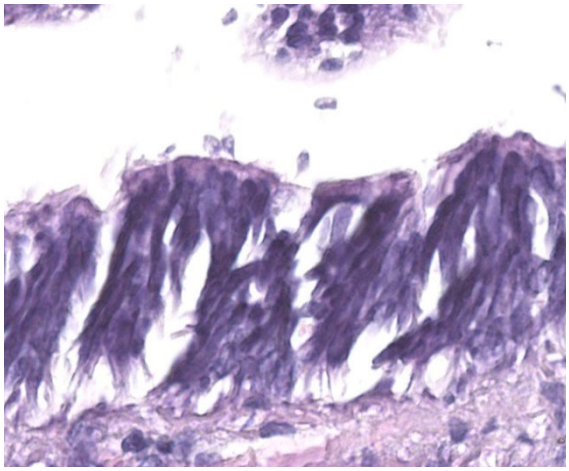

Figure 1-4. Gill lamellae, octopus. Ichthyobodo sp. are attached to the hyperplastic gill epithelium. (HE, 890X)

layered dark membrane. In some cases, abundant developmental stages may be accompanied by desquamation and necrosis of the gill epithelium.

Detection of coccidiosis may be particularly important in octopods caught from the wild for rearing in aquaculture or exhibition in aquariums. Fecal oocyst count is the most common method of detection, although there are no standardized ranges of normal recognized in cephalopods. Determination of the species relies on the morphological characterization of the size, shape, and number of sporozoites in each sporocyst, as well as the ornamentation.[2] For example, *Aggregata dobelli* has a smooth-surfaced sporocyst containing 9-22 sporozoites. The positive identification of this developmental stages requires histological examination of the digestive tract, which cannot be performed in living animals. Treatment also presents a significant dilemma. While protozoan infections in aquarium species are typically controlled through the addition of ionic or chelated copper, high concentrations of formalin, or chloroquine diphosphate solutions to closed tanks, these methods are unsuitable for use on octopods.[1] Thus, the ongoing study of coccidiosis in cephalopods requires new methods of both molecular detection and eradication of these parasites.

Contributing Institution:
Johns Hopkins School of Medicine Department of Molecular & Comparative Pathobiology (https://mcp.bs.jhmi.edu/)

JPC Diagnosis:
Gill: Branchitis, hemocytic, diffuse, moderate, with numerous intraepithelial macrogametocytes (etiology consistent with *Aggregata* sp.) and few surface flagellates (etiology consistent with *Ichthyobodo* sp.).

JPC Comment:
The contributor provides an excellent overview of *Aggregata,* a very important pathogen of octopuses. Another organism featured in this case is *Ichthyobodo*, a free swimming flagellate which can infect freshwater and marine fish and octopuses.[7] The flagellate, which measures 6-10 µm, attaches to and penetrates epithelial surfaces, especially the gills.[7] Gross lesions are typically minimal, and histologic lesions range from absent to severe bronchitis with epithelial degeneration and necrosis.[6,9] The parasites may not be seen histologically as they quickly depart the host after it dies.[9] In a recent survey of 19 wild-caught giant Pacific octopuses which died captivity, ten of the animals had *Ichthyobodo* infections, nine of which had severe bronchitis.[6] In this case, *Ichthyobodo* is present in low levels and was probably not pathogenic to the patient.

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CASE II:

Signalment:
An adult, captive, female giant spider crab (*Macrocheira kaempferi*; Temminck, 1836).

History:
This giant spider crab (*Macrocheira kaempferi*) was found deceased after a short period of anorexia. Other decapods in the tank had shown signs of "shell disease syndrome".

Gross Pathology:
At necropsy, the main gross pathologic findings were multifocal, variably sized, dark brown to black, depressed skin foci in the apron, limb, and carapace

Laboratory Results:
Multiple bacterial cultures of skin and internal organs from other similarly affected and deceased crabs yielded *Vibrio* sp. (1+), *Shewanella putrefaciens* (1+), and *Corynebacterium* sp. (1+).

Microscopic Description:
Apron, Carapace, Limb: Multifocally, the epi-, exo- and endo-cuticle as well as the membranous layer are eroded and ulcerated. Variable amounts of proteinaceous material and hemocyte debris expand and disrupt the cuticle, membranous layer, and epidermis. Multifocally, the epidermis and dermis are infiltrated by granular and reactive agranular hemocytes, occasional melanized cells, karyorrhectic cellular debris and extracellular and intraphagocytic bacteria. Inflammatory

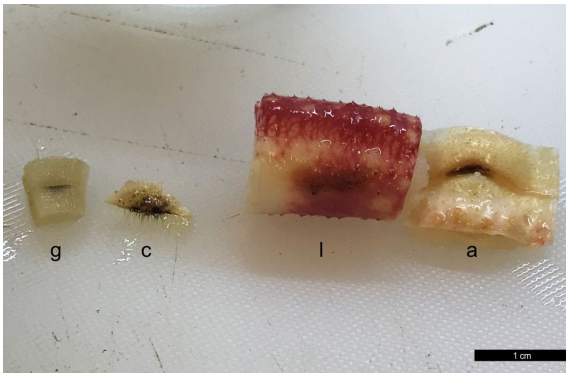

Figure 2-1. Carapace, spider crab. There are multifocal, dark brown to black, depressed skin foci in the apron, limb, and carapace. (Photo courtesy of: Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL), 483 Agronomy Rd, College Station, TX 77843, USA. https://tvmdl.tamu.edu/)

infiltrates are often more prominent at the setae. Some of these hemocytes infiltrate the connective tissue amid myocytes and variably infiltrate and disrupt vascular structures, more prominently within the deep soft tissue in the apron. Rare hemocyte nodule formation is noted. Hemolymph sinuses exhibit occasional eosinophilic globules.

Gill: Multifocally, the gills are infiltrated and disrupted by granular and agranular hemocytes, cellular debris, proteinaceous fluid, and intraphagocytic and intravascular bacteria.

Contributor's Morphologic Diagnoses:
Apron, Carapace, Limb: Mild to marked, multifocal, subacute erosivo-ulcerative hemocyte epidermitis and dermatitis/hypodermitis with intralesional and intravascular gram-negative bacteria, melanization, and vasculitis.

Gill: Moderate, multifocal, subacute hemocyte branchitis with intralesional and intravascular gram-negative bacteria.

Contributor's Comment:
Based on the clinical history and bacterial culture results from similarly affected and deceased conspecifics, the gross and histopathologic findings in this crab are strongly suggestive of black spot shell disease syndrome (SDS) and hemocoelic bacterial invasion (septicemia).[3,12,15]

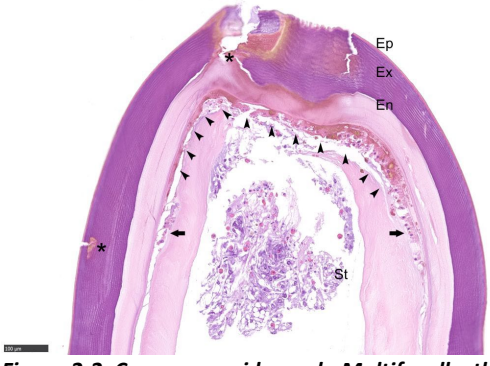

Figure 2-2. Carapace, spider crab. Multifocally, the epi-, exo- and endo-cuticle as well as the membranous layer are eroded and ulcerated. (Photo courtesy of: Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL), 483 Agronomy Rd, College Station, TX 77843, USA. https://tvmdl.tamu.edu/)

Macrocheira kaempferi (infraorder Brachyura, order Decapoda) is the largest of extant Arthropoda. This species occurs along the Pacific coast of Japan, at depths ranging from 50 to 400 m.[5] Although *M. kaempferi* has been introduced into aquaria worldwide, knowledge on health and disease aspects in the species is very limited.[1,8]

SDS is regarded as a multifactorial syndrome and is one of the main concerns on crabs newly introduced into aquaria.[1,9] Two potential forms of SDS have received attention. These include a) the "classic" form, which is centered around chitin degradation and has been reported in many crustaceans, including lobsters, crabs and shrimp,[7,13] and b) an "epizootic" form, in which chitin degradation is of less significance and primarily affects lobsters.[2] Various environmental stressors[14] and opportunistic chitinolytic bacteria (particularly in the classic form), such as *Vibrio* sp, *Photobacterium* sp, *Aeromonas* sp, *Alteromonas* sp, *Pseudoalteromonas* sp, *Clostridium* sp, *Cytophaga* sp, *Chromobacteria* sp., protozoans and/or fungi play central roles.[4,14,10] High occurrence is linked to polluted environments.[14]

Grossly, SDS is characterized by exoskeletal erosions, pitting, and discoloration, including typical black spots resulting from melanization.[4] Lesions vary from mild (restricted to the exoskeleton), to severe, where injury may extend through the entire shell and into the soft tissues, often resulting in extensive ulcers, fractures, and autotomy.[6] Lesions may initiate via destruction of the epicuticular layer, by proteolytic and lipolytic microbial activities, predatory or cannibalistic attacks,

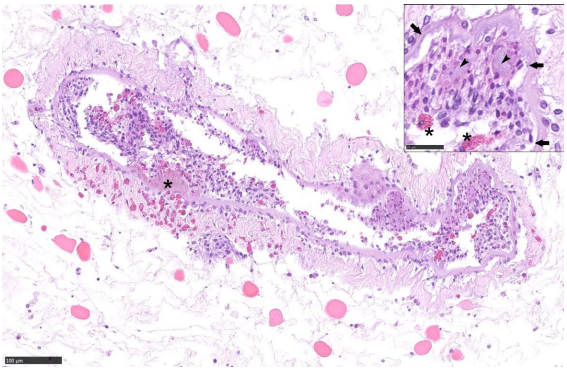

Figure 2-3. Carapace, spider crab. Hemocytes infiltrate the connective tissue, myocytes vascular structures, more prominently within the deep soft tissue in the apron. (Photo courtesy of: Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL), 483 Agronomy Rd, College Station, TX 77843, USA. https://tvmdl.tamu.edu/)

chemical injury or the abrasive action of sediment and/or articulated body parts; the setal pores are often affected first and may favor internal invasion.[7] Microscopically, there is progressive erosion, ulceration and disruption of the exoskeleton and epidermis. The inflammatory response may include exudation of proteinaceous material, hemocyte debris, infiltration of granular and reactive agranular hemocytes, melanized cells, and extracellular and intraphagocytic bacteria; these changes were observed in the present case. In severe cases, bacteria may extend into the internal viscera and mortality can ensue as result of primary or secondary infection.[12] In the present case, traumatic skin injury by conspecific males likely predisposed to opportunistic bacterial colonization, resulting in eventual hemocoelic bacterial invasion, septicemia, and death.[11,12]

SDS is difficult to control and eradicate, despite occasional remission after molting.[3] Although fallible, treatment methods have been proposed to contain disease progression.[1] In conclusion, the diagnosis of SDS relies upon visualization of typical gross findings combined with histopathology and microbiologic analysis.

Contributing Institution:
Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL), 483 Agronomy Rd, College Station, TX 77843, USA. https://tvmdl.tamu.edu/

JPC Diagnosis:
Exoskeleton and gills: Dermatitis and branchitis, ulcerative, hemocytic, chronic, multifocal, moderate, with melanization and cuticular epithelial hyperplasia, vasculitis, and intraphagocytic and extracellular bacteria.

JPC Comment:
This week's moderator, Dr. Elise LaDouceur, editor of the recently published textbook *Invertebrate Histology*, described the invertebrate immune system, which lacks an adaptive arm and features hemocytes as the effector cell in most species. In crustaceans and other arthropods, hemocytic recognition of pathogen- or damage-associated molecular patterns leads to adhesion, degranulation, and phagocytosis, or for larger pathogens, encapsulation or nodulation with subsequent melanization. The last step leads to formation of reactive oxygen and nitrogen species and is the underlying cause of the eponymous "black spots" in this syndrome.

Chitin is a biopolymer which makes up 70% of the organic fraction of crustacean exoskeletons and is rapidly degraded by microbes after crustacean molting or death.[14] Chitinolytic bacteria travel along a chitin oligosaccharide gradient, adhere to and form biofilms on chitinous surfaces, express chitin catabolic cascade genes, and produce chitinase and beta glucosaminidases responsible for chitin breakdown.[14] These mechanisms allow the bacteria to use chitin as a source of nitrogen and carbon, which are absorbed through chitoporins.[14]

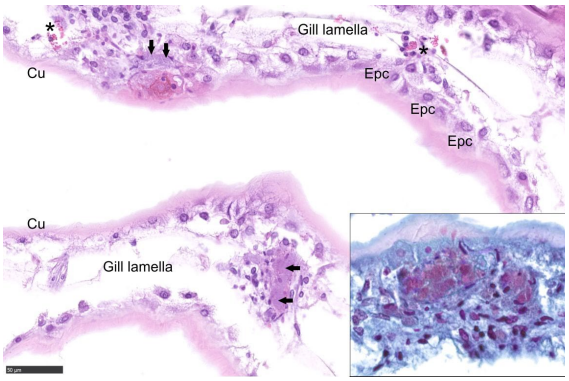

Figure 2-4. Carapace, spider crab. Gills are infiltrated a by granular and agranular hemocytes, cellular debris, proteinaceous fluid, and intraphagocytic and intravascular bacteria. (Photo courtesy of: Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL), 483 Agronomy Rd, College Station, TX 77843, USA. https://tvmdl.tamu.edu/)

The healthy cuticle of crustaceans is resistant to such chitinolytic activity because the most superficial layer – the epicuticle – is nonchitinous and composed primarily of proteolipid material.[12] Disruption of the epicuticle may be mediated by trauma or abrasion, as mentioned by the contributor, or by lipolytic actions of other microbes.[12] Older crustaceans are more susceptible to degradation as they molt and replace their exoskeleton less frequently.[14] Once the epicuticle is disrupted, the chitinous pro-cuticle is exposed and can be colonized by chitinolytic bacteria, which are usually part of a mixed population of microbes which may produce other enzymes, scavenge liberated nutrients, or prey on other microbes.[14]

As the contributor describes, severe lesions can lead to secondary hemocoelic invasion and septicemia. Internal lesions associated with SDS were previously described in a study of shell-disease affected edible crabs, *Cancer pagarus*.[12] In the gills, there were hemocytic infiltrates and nodules which frequently occluded the hemal sinuses, the thin epithelium was occasionally breached and sealed by melanized hemocytic plugs, and nephrocytes at lamellar tips were swollen with abundant dark brown material within a central cytoplasmic vacuole.[12] Hepatopancreatic tubules exhibited variable necrosis and hemocytic nodules in hemal spaces.[12] Hemocytic nodules were also observed in the heart.[12] In general, the severity of the internal lesions mirrored the extent of the shell lesions.[12]

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CASE III:

Signalment:
5 year old, female, African spurred tortoise, *Geochelone* (*Centrochelys*) *sulcata*, *C. sulcata*

History:
Animal kept as pet by a private. No information regarding general state of health, past medical history and gross *post mortem* findings were given by the referring private veterinary practitioner. Only a formalin fixed liver was submitted for histology.

Gross Pathology:
The liver was moderately increased in volume, with rounded margins and diffusely brown to yellow in color. On the surface and in cross-section, multiple to confluent, round and white to yellow lesions was present (multifocal necrotizing hepatic foci). The lesions are smooth with moderately irregular margins and are surrounded by a hyperemic halo. In cross section, hemorrhages are present in the center of the necrosis. The consistency of the liver is markedly reduced, and the organ is friable to the manipulation.

Laboratory Results:
No findings reported.

Microscopic Description:
Liver: Approximately 30% of the hepatic parenchyma is characterized by multifocal, random, 1-3 mm in diameter, irregularly nodular, areas of necrosis and heterophilic-histiocytic inflammation.

The centre of the lesions is composed of abundant basophilic granular karyorrhec-

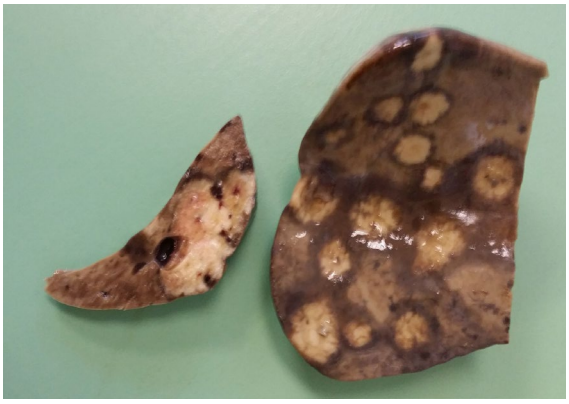

Figure 3-1. Liver, tortoise. There are multifocal to coalescing areas of necrosis within the liver. (Photo courtesy of: DIMEVET-Anatomical Pathology Section, University of Milan.)

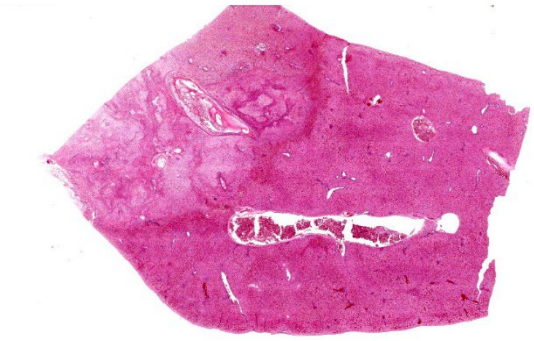

Figure 3-2. Liver, tortoise. A large area of coagulative necrosis rimmed by an area of hemorrhage and necrosis is present at left. (HE, 5X)

tic/necrotic debris with complete loss of cellular details (lytic necrosis) admixed to intensely eosinophilic, shrunken hepatocytes with lysed nuclei (coagulative necrosis). Elevated numbers of both viable and degenerated heterophils with karyolitic nuclei can be seen admixed with the necrosis and at the periphery of the necrotic foci that are multifocally also associated at the periphery with a lightly eosinophilic fibrillary, finely beaded, meshwork (fibrin).

At the periphery of the necrotic foci, surrounding and invading vessel walls and occasionally in the cytoplasm of macrophages variable numbers of oval (15-30 µm in diameter) protozoal structures can be seen. Parasites have a distinct cell membrane, a finely granular to vacuolated cytoplasm, and a single nucleus with marginated chromatin and a lightly basophilic central karyosome (amoebic trophozoites).

The hepatic parenchyma is multifocally characterized by swollen hepatocytes, with granular to clear cytoplasm (vacuolar/hydropic degeneration). Multifocally in the cytoplasm of hepatocytes, yellow-greenish granular pigment (bile stasis) is evident.

Fibrin thrombi occasionally entrapping trophozoites are visible in vascular lumens with endothelial cell necrosis. Diffusely sinusoidal hyperemia is present.

Contributor's Morphologic Diagnoses:
Liver: severe, multifocal to coalescing, random, acute to subacute necrotizing hepatitis and vasculitis with perivascular and intralesional trophozoites consistent with *Entoamoeba* spp.

Contributor's Comment:
Entamoeba invadens is the most important amoeba species infecting reptiles.[6,14,18] This protozoan parasite belongs to the phylum *Sarcomastigophora*, subphylum *Sarcodina (Rhizopoda)*, order *Amoebida*, family *Entamoebidae*, genus *Entamoeba* and is similar morphologically to *Entamoeba histolytica*. Although, *E. invadens* may be the main parasite involved in reptile pathology, other *Entamoeba* species, such as *E. terrapinae, E. insolita, E. barreti, E. testudini*s and *E. ranarum,* can also infect reptiles[8].

E. invadens is a common commensal parasite of different free-ranging reptiles including snakes, crocodilians, turtles, tortoises, and lizards.[18] Turtles, tortoises, and crocodiles are considered reservoir species for *E. invadens*. Herbivorous tortoises harbor the parasite and only occasionally demonstrate clinical signs.[6,11] The mechanism of this resistance is unknown. Based on data available for *Acanthamoeba*, it is possible that some types of sugars (mannitol and glucose) can prevent the adhesion of the trophozoite to the mucosal surface. Among tortoises, giant tortoises (*Geochelone* spp.)[11] and the northern map turtle (*Graptemys geographica*)[17] seem to be more susceptible. Snakes which develop amebiasis are *Boidae* (boas), *Pythonidae* (pythons), *Crotalidae* (crotalids), *Elapidae* (colubrids), and *Viperidae* (vipers). Snakes resistant are greater snake and northern black racers. All the crocodile seems to be immune to *E. invadens.*[4] The presence of carnivorous reptiles resistant to the infection has been associated with the evolutionary strategy to feed with snakes.[4] Among lizards,

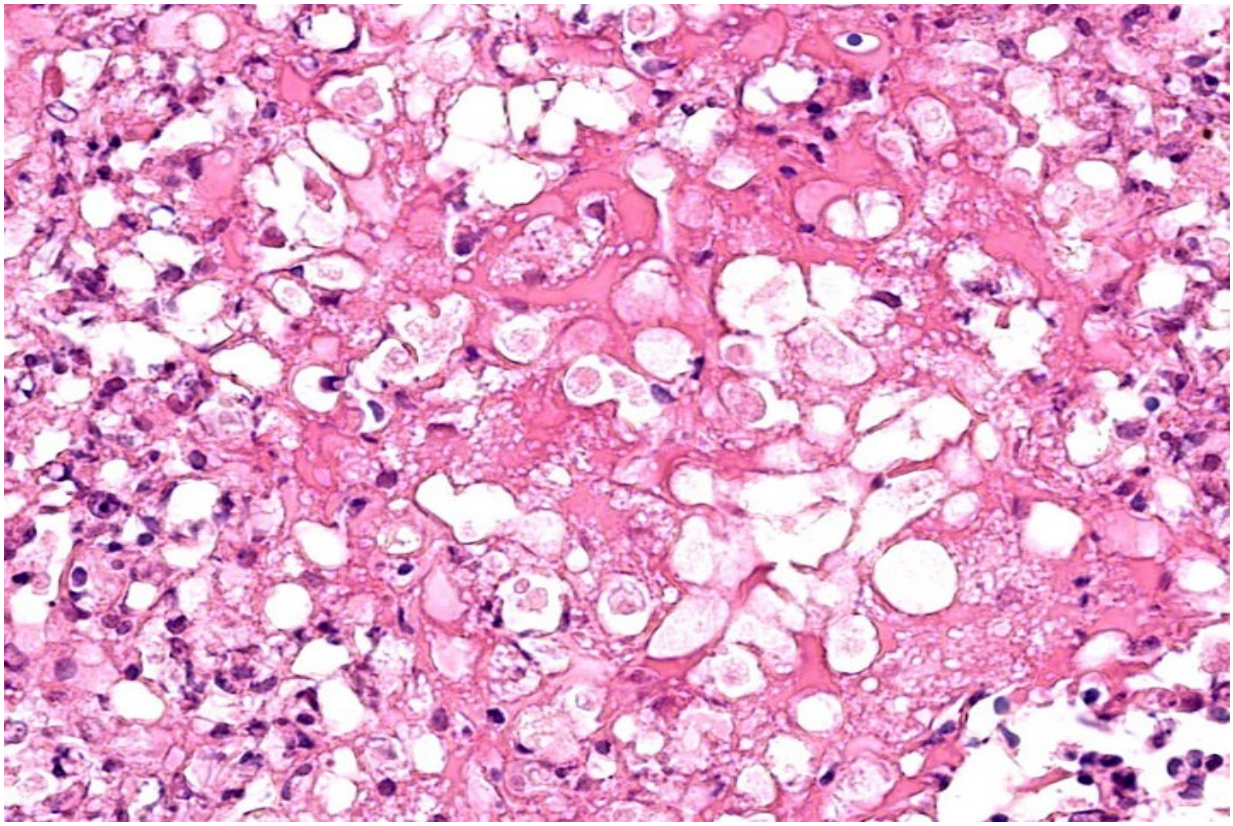

Figure 3-3. Liver tortoise. Areas of necrosis contain numerous extracellular amebic trophozoites. (HE, 280X)

Varanidae (monitor lizard) seems to be the most susceptible species. The onset of clinical signs in resistant animals has been correlated with stress, age and immunosuppression.[8]

Hepatic amoebiasis in tortoises is rare and an outbreak of severe entero-hepatic disease with duodenal involvement seems to have been reported once.[9] In snakes and lizards, *E. invadens* is responsible for a severe epizootic disease characterized by necro-hemorrhagic gastroenteritis, colitis, or entero-hepatitis. Occasionally, the parasite can invade the bloodstream and reach other organs causing extraintestinal complications. Atypical amebiasis manifesting as myositis and ulcerative dermatitis has been reported in a common water monitor lizard (*Varanus salavtor*).[2]

E. invadens has a worldwide distribution[2], with a life cycle characterized by two morphologically different stages, a motile trophozoite stage (vegetative form) and a cystic stage (resistant form). Because of the protection conferred by their walls, amebic cystic forms can survive days to weeks in the environment. In asymptomatic carriers, trophozoites are confined to the intestinal lumen (non-invasive infection). In some animals, trophozoites acquire invasiveness, infiltrating the intestinal wall and the biliary ducts, inducing the enteric form (intestinal disease) or the systemic form (extraintestinal disease). In snakes and lizards, the liver is considered the major extraintestinal target organ.[7] In snakes, invasion of lungs, spleen, pancreas, kidneys, and subcutaneous tissues occurs via hematogenous route.[9]

The èathogenic transformation of the trophozoite into an invasive parasitic form has not been elucidated for *E. invadens*. Although a parallel with the pathogenesis of *E. histolytica* in human beings has been hypothesized

explaining the different forms via the recognition of different parasite strains.[19]

The parasite has an oro-fecal cycle, mature amoebal cyst are ingested with contaminated water, food or arthropod vectors (flies, roaches). Excystation occurs in the small intestine with the release of a trophozoite characterized by four nuclei that after division produce four trophozoites with a single nucleus. Each of these 4 zoites divides in 2, producing a total of 8 trophozoites (amebulae). Trophozoites are the mobile and labile form of the parasite and are capable of migrating in the large intestine. Proteolytic enzymes (glycosidases such as galactosidase, mannosidase, fucosidase and others) produced by trophozoites disrupt the mucous barrier and parasites are able to attach to the gastrointestinal epithelium. The trophozoite first adheres to the intestinal mucin layer and to epithelial cells by a surface Gal/GalNAc-specific lectin (adhesin), and releases pore-forming polypeptides called amoebapores that are small potent peptides able to induce lysis of host cell membrane resulting in intracellular calcium elevation and eventual cell death. After the epithelial cells are lysed, there is colonic gland invasion and cysteine proteases degrade the extracellular matrix so that trophozoites burrow into lamina propria invading the submucosa[5.] In the large intestine trophozoites start dividing by binary fission producing cysts released in the environment. In humans, amoebic liver abscess (amoeboma, ALA=Amebic liver abscess) is the most frequent extraintestinal manifestation of *E. histolytica* infection. The liver can be infected by two ways, by common biliary duct invasion (continuity ascending route), or by portal vein invasion (thromboembolic route).

Virulence factors involved in ALA development include those necessary for complement resistance (PPGs), ROS resistance (peroxiredoxin), lysis (CPs and amoebapores), and cell adherence (notably, KERP1 and the Gal/GalNAc lectin).[17]

In a recent study on *E. histolytica* pathogenesis, amoebae were demonstrated to kill by biting off and ingesting distinct fragments of viable cells. The internalization of bites of living human cells is reminiscent of trogocy-

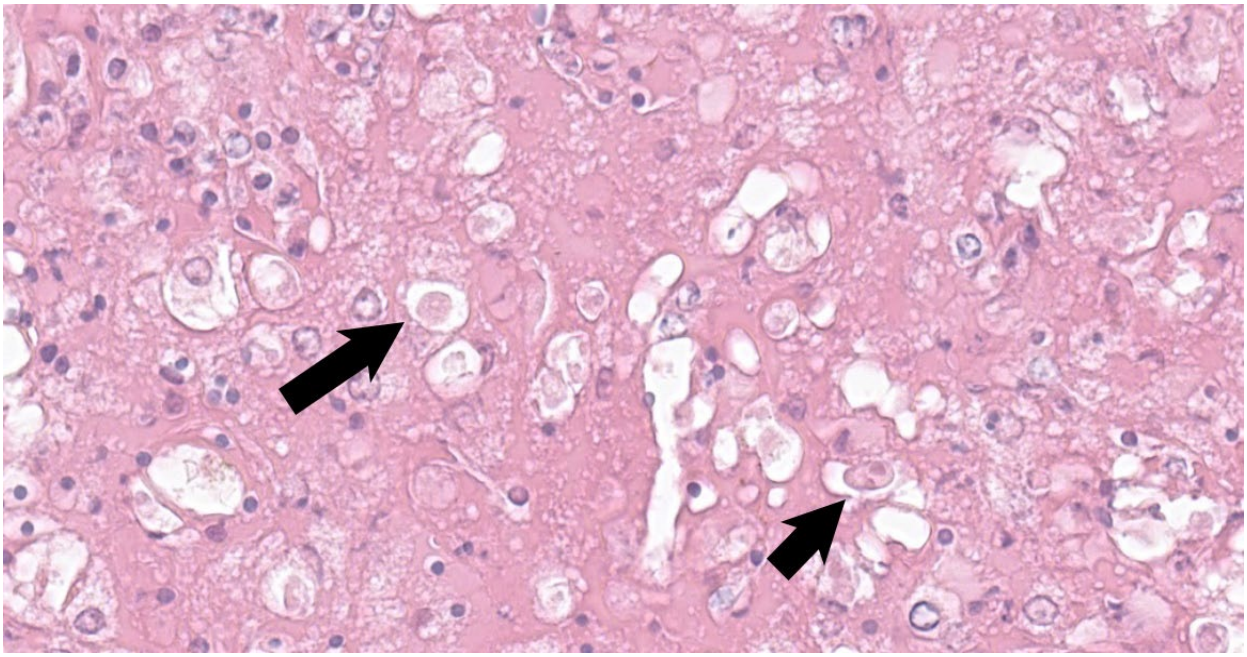

Figure 3-4. Liver, tortoise. Higher magnification of trophozoites (arrows). (HE, 527X)

tosis (Greek *trogo–*, nibble) observed between immune cells, but amoebic trogocytosis differs since it results in death.[13]

Clinical signs associated with amoebiasis in reptiles include anorexia, weight loss, mucoidal or hemorrhagic diarrhea, dehydration, and death after weeks or month of illness. Sudden death is also commonly reported.[4,16,11]

Typical gross findings are thickening of the gastrointestinal wall, with ulcerative and necrotizing colitis in snakes and duodenitis in turtles. Lesions can extend to other parts of the gastrointestinal tract.[2,9] Tubular organs can have transmural ulceration. Ulcers may be flask shaped, with a narrow neck and broad base. The lumen of the intestine is filled with blood, necrotic debris, and mucus. Extraintestinal lesions are secondary to the intestinal infection, and all organ systems can potentially be affected. The lesions start as abscesses evolving in necrotizing lesions centered on blood vessels.[2] Secondary bacterial infections are commonly found in liver and gastrointestinal tract.

Typical histological findings are necro-hemorrhagic lesions in all organs affected. Usually, amoebae can be found admixed to the necrotic debris. *E. invadens* trophozoites range from 10 to 21 µm (intestine: 12-19 x 10-13 µm, liver: 13-21 x 11-18 µm).[9] Nucleus is 3-5 µm in diameter, round and periphery located. A single central endosome is present, and it is 0.6-1 µm. *E. invadens* cysts range from 11 to 20 µm and have four nuclei.

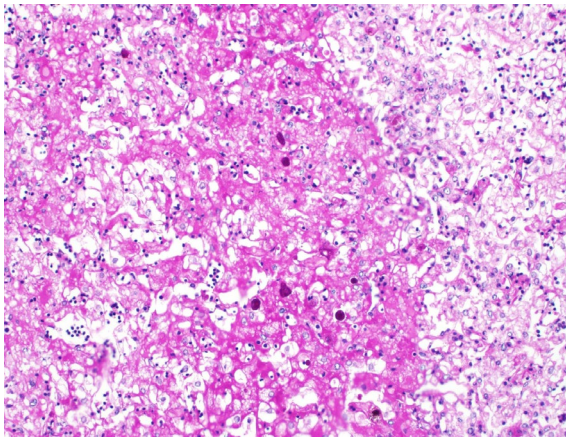

Figure 3-5. Liver tortoise. Amebic trophozoites are densely PAS-positive. (PAS, 200X)

Ultrastructurally,[3] trophozoite cytoplasm is filled by numerous, variably sized vacuoles. Vacuoles are surrounded by an electron dense membrane and contain starch granules and bacteria (food vacuoles). Cytoplasmic, irregularly shaped, electron dense aggregates of 200 nm are associated with the food vacuoles and the nucleus (chromatoid particles). Inside the nucleus, a single electron dense nucleolus is usually present. Cyst cytoplasm can be divided into two areas, one electrondense adjoining the nuclei and around the cell wall and containing vacuoles, and one electrolucent, in between, containing glycogen. The chromatoid particles present in the cyst, are usually aggregated in large crystalline structures (chromatoid bodies).[3]

Gross and histopathological finding are distinctive and mostly diagnostic, additional diagnostic tools are: fecal flotation, histochemical stains (PAS staining-purple, GMS staining-black, and Heidenhain's iron strain for chromatin), immunohistochemistry, indirect immunofluorescence and PCR.[1,18]

Other infectious agents causing hepatic necrosis and hepatitis in tortoises include: Herpes viruses[10] (i.e. tortoise herpesvirus type 1 and 2- responsible for sudden death, upper respiratory disease, hepatic disseminated necrosis and intranuclear eosinophilic inclusion bodies; siadenovirus[16] (systemic infection, intranuclear basophilic to amphophilic inclusion bodies in hepatocytes and non suppurative hepatitis), Iridoviruses (i.e. gen. Ranavirus: disseminated hepatic necrosis, basophilic inclusion bodies, vasculitis)[10]; Bacteria, mostly in septicemic infections or following a primary viral disease (i.e. *Aeromonas*

spp. Escherichia coli, Pasteurella testudinis, Morganella morganii, Serratia marcescens Chlamydophila spp.[10] and Fungi causing sistemic mycoses such as *Aspergillus, Paecilomyces,* and *Penicillium* spp. can sporadically induce hepatic lesions.[10]

Contributing Institution:
DIMEVET-Anatomical Pathology Section
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JPC Diagnosis:
Liver: Hepatitis, necrotizing and embolic, focally extensive, marked, with vasculitis and numerous amoebic trophozoites.

JPC Comment:
A variety of pathogenic and commensal *Entamoeba* spp. infect the gastrointestinal tract of vertebrates and invertebrates, and the contributor provides a thorough summary of *Entamoeba* infection in reptiles. In addition to *E. invadens,* snakes may rarely be susceptible to another *Entamoeba* species – *Entamoeba ranarum*, which typically infects but rarely causes disease in amphibians.[12,14] There have been two published reports of *E. ranarum* infection causing necrotizing colitis in snakes; one in a ball python, and one in a boa. While both cases had colitis similar to that caused by *E. invadens,* neither featured spread to other organs, such as the liver.[12,14] In one case, the snake had previously been housed with frogs, so fecal-oral transmission is hypothesized. [12]

Another *Entamoeba* afflicting amphibians has recently been identified in invasive cane toads (*Rhinella marina*) in Australia. These amphibians were introduced in 1935 as an unsuccessful means to control beetles consuming sugar cane crops and now inhabit a range of over 1 million square kilometers.[16] In 2014, researchers discovered the novel *Entamoeba* during an outbreak of severe colitis and high mortality in cane toads in one region of northern Australia. PCR and subsequent sequencing illustrated its close relation to both *E. ranarum* and *E. invadens* and lead to its classification is *Entamoeba* sp. CT1.[20] Affected toads had either no gross lesions or mild prominence of vasculature and thickenning of intestinal walls. Histologically, all affected toads had some degree of colitis, with the most severe cases featuring deep ulcers with edema and fibrosis. *Entamoeba* trophozoites were associated with the affected mucosal epithelium and ulcers, but there was no evidence of spread outside the gastrointestinal tract.[20] Subsequent research to evaluate the impact on native frog species has demonstrated a prevalence of approximately 24% in cane toads in the same region and no identification of the parasite in any of the 11 tested native frog species.[16] While the authors acknowledge that intermittent shedding, sample degradation, and testing limiations may have affected the abiilty to isolate the parasite, the results are encouraging for native species and anuran conservationists.[16]

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CASE IV:

Signalment:
9-year-old, female, Crawl Cay Boa (*Boa imperator*)

History:
This animal was the only affected animal from a small, multi-species, zoological collection which presented with anorexia for the preceding 7 months, with gradually progressive weight loss resulting in a thin body condition at presentation.

Gross Pathology:
There was a 20cm x 10cm fluid filled structure within distended the mid-coelomic cavity which corresponded to an approximately 20cm long segment of jejunum which was markedly dilated up to approximately 10cm diameter. At this site, on the intestinal mucosal surface were numerous, white, raised nodules measuring up to approximately 1mm x 5mm which were interspersed by multifocal, firm, larger masses measuring up to 2cm diameter and forming multifocal papilliform projection which protruded into the intestinal lumen. At the aboral end of this segmental dilation there was an approximately 10 cm long segment of the intestine which was distorted and had 'telescoped' into the more distal intestine, forming an intussusception.

Laboratory Results:
Immunohistochemistry: CD3 negative, CD20 negative and Iba-1 positive. In-situ hybridisation: Boa CD20 negative.

Microscopic Description:
The intestine at the level of the intussusception was markedly expanded and transmurally effaced by an expansile, multilobulated, unencapsulated and poorly demarcated proliferation of neoplastic, monomorphic round cells forming sheets supported by a fine fibrovascular stroma. These variably formed frond like projections, coalescent polypoid masses or locally expanded the lamina propria. These cells were up to approximately 25 µm diameter (3-4 x the diameter of an erythrocyte), with distinct borders and a large amount of eosinophilic cytoplasm. The nuclei were round to oval, central to eccentrically positioned, with euchromatic and stippled to vesicular chromatin and frequent large nucleoli. There was moderate anisocytosis and anisokaryosis with 10 mitotic figures per 2.37 mm² (equivalent to 10 high power fields; hpf; x400 magnification), some of which were bizarre in form. Occasionally, in multifocal areas, these cells were expanded by increased, clear intercellular spacing (intercellular oedema). The epithelium was multifocally ulcerated with replacement by eosinophilic, homogenous material (fibrin), degenerate heterophils, sloughed necrotic epithelial cells, eosinophilic and karyorrhectic debris, and swathes of basophilic. In other areas, there was slight piling up of epithelial nuclei (hyperplasia; attempted regeneration).

Contributor's Morphologic Diagnoses:
Intestinal histiocytic sarcoma with secondary intussusception.

Contributor's Comment:
The features in this consisted of a coelomic dilation corresponding to a large intestinal dilation and associated intussusception. Microscopic examination revealed extensive effacement of the intestinal mucosa by sheets of neoplastic round cells, which were strongly Iba-1 positive, indicative of a histiocytic sarcoma at this site. Considering the lack of additional lesions, this intussusception was most likely secondary to this neoplasm.

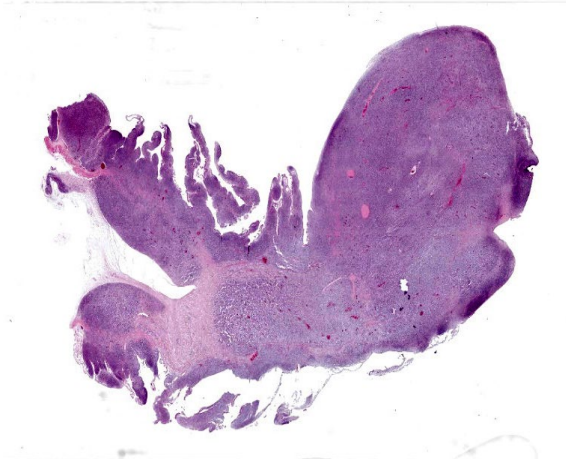

Figure 4-1. Intestine, boa. One section of intestine, markedly expanded by a round cell neoplasm, is submitted for examination. (HE, 5X)

Intestinal intussusception is clinically defined as the telescoping of one segment of digestive tract into the lumen of an adjacent segment, forming an inner intussusceptum and surrounding intussuscipiens. This forms a distended segment of intestine, with significant vascular compression resulting in progression to ischaemic necrosis of both the intussusceptum and intussuscipiens

Histiocytic sarcoma itself has only been rarely reported in boa species, with a single case identified in both a rosy boa (*Lichanura trivirgata*) and a boa constrictor (*Boa constrictor*), whilst an additional single histiocytoma was identified in a rainbow boa (*Epicrates cenchria*).[4,5] Similarly, histiocytic neoplasms have been only rarely identified in other snake species including single reports in a common garter snake (*Thamnophis sirtalis*) and bull snake (*Pituophis catenifer sayi*), though it was not stated in these cases if any additional confirmation such as immunohistochemistry was performed.[5,15]

Only a small number of published case reports have described intussusception secondary to other neoplasms in snakes, including T-cell lymphoma and myeloid leukaemia in boa constrictors and a non-characterised round cell tumor in an Australian sea snake (*Hydrophis major*).[6,8,13] Similarly, only small numbers of published reports describe non-neoplastic causes of intussusception in snakes, including idiopathic intussusception in a pine snake (*Pituophis melanoleucus*) and intussusception secondary to cryptosporidiosis in a corn snake (*Pantherophis guttatus*).[1,16]

Differentiation of histiocytic tumors from other round cell lineages is often challenging in snakes, particularly in comparison to lymphoid proliferations, which often share similar morphological features.[5] Furthermore, consistent immunohistological phenotyping of neoplasms is challenging in reptiles due to the lack of established antibody markers, preventing accurate and reliable immunophenotyping.[13]

Intussusception is a rare but recognized complication of round cell tumors in other species, including humans.[2,9] Similarly to veterinary species, there are only limited individual case reports in human medical literature

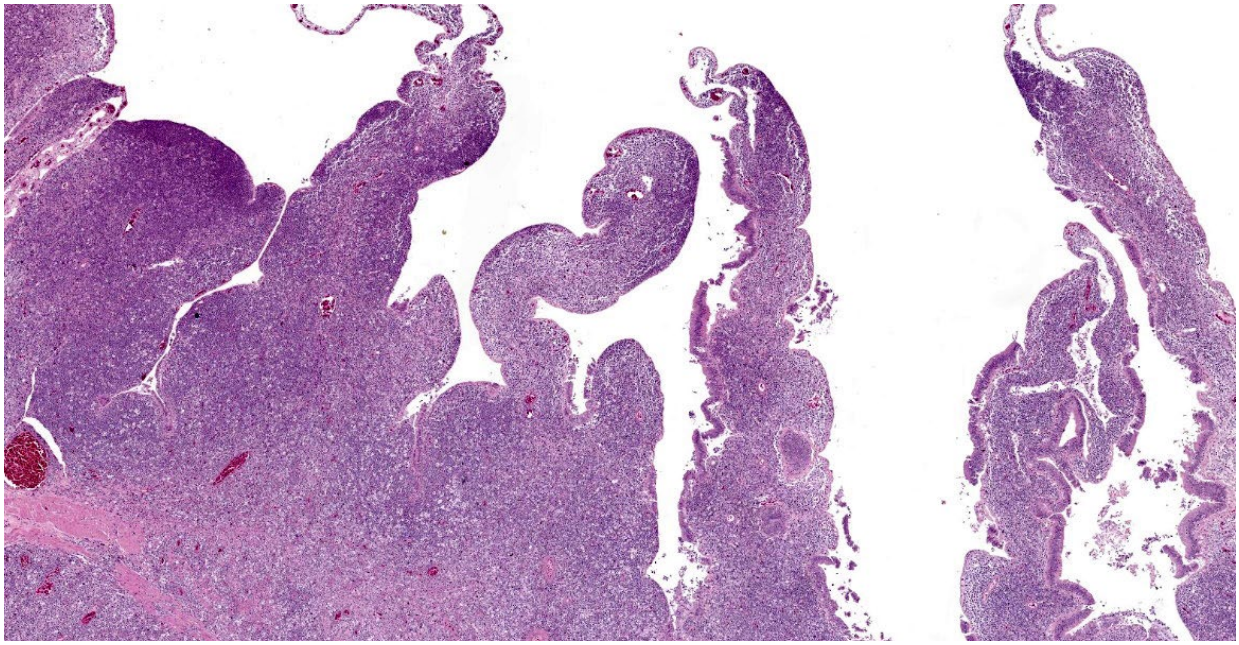

Figure 4-2. Intestine, boa. Villi are markedly expanded by sheets of neoplastic round cells. (HE, 29X

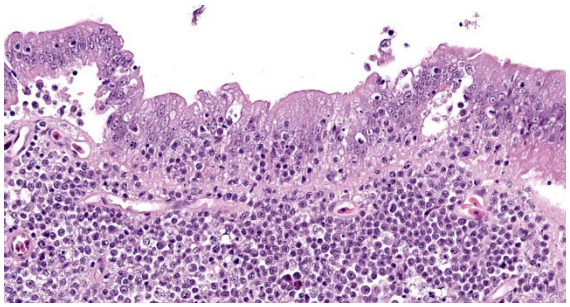

Figure 4-3. Intestine, boa. Small to moderate numbers of neoplastic cells infiltrate the overlying remnant mucosal epithelium. (HE, 381X)

of histiocytic sarcoma associated intussusception.[12]

Contributing Institution:
Easter Bush Pathology – Royal (Dick) School of Veterinary Studies. https://www.ed.ac.uk/vet/services/easter-bush-pathology

JPC Diagnosis:
Intestine: Lymphoma.

JPC Comment:
While a less specific diagnosis of round cell tumor was considered, conference participants ultimately favored a diagnosis of lymphoma based on the histologic appearance of neoplastic cells which had scant cytoplasm and a generally round nucleus. Immunohistochemical evaluation conducted by the JPC was consistent with B-cell lymphoma. Most neoplastic cells had strong neoplastic immunoreactivity for PAX-5, a B cell marker, with few infiltrating T cells positive for CD-3 and IBA-1 highlighting few cells with dendritic morphology, consistent with tumor associated macrophages. Dr. LaDouceur, this week's moderator and author of the Reptile Neoplasia chapter in *Noninfectious Diseases and Pathology of Reptiles,* explained that successful B cell markers are difficult to find in reptiles, and PAX-5 and BLA-36 provide the most utility. The presence of few cells with basophilic cytoplasmic granules lead to brief consideration for a metastatic chromatophoroma; however, there was metachromatic staining to the granules on Giemsa, identifying the cells as mast cells and likely part of inflammation secondary to mucosal ulceration.

In several studies of neoplasms in snakes, malignancies far outnumber benign tumors, ranging from 70 to 80% of all diagnosed neoplasms.[3,10,15] In general, hematopoietic neoplasia, specifically lymphoma, is the most common neoplasm in snakes, though there is some variation between reports, presumedly due to variation in species composition in various collections.[7,10,15] As in other species, lymphoma in snakes may be multicentric and form discrete masses or diffuse organomegaly with or without a leukemic component.

The histomorphology may appear plasmacytoid or histiocytoid, making immunohistochemical often necessary to confirm an uncommon diagnosis of histiocytic sarcoma.[7] Other common neoplasms in snakes include soft tissue sarcomas, renal adenocarcinomas, fibrosarcomas, and melanomas.[5]

Another differential to consider cases of histiocytic or granulomatous inflammation in

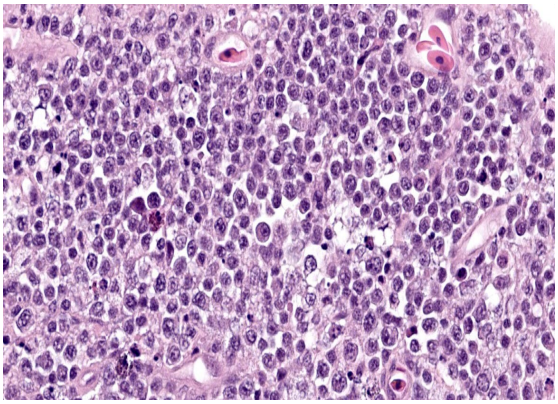

Figure 4-4. Intestine, boa. High magnification of neoplastic round cells. (HE,648X)

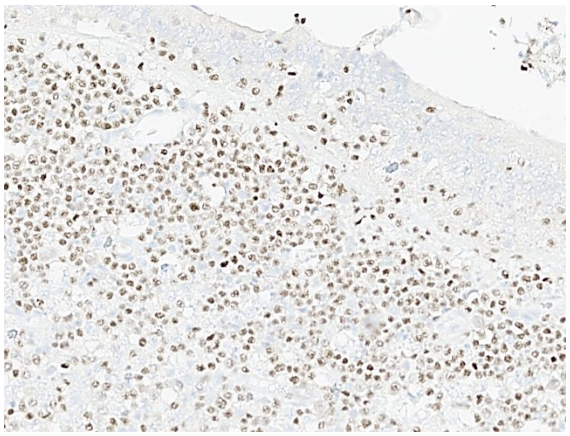

Figure 4-5. Neoplastic cells demonstrate strong nuclear immunoreactivity for PAX-5. (anti-PAX-5, 276X)

snakes is atypical or nontuberculous *Mycobacterium* infection, which causes histiocytic, granulomatous, or occasionally heterophilic inflammation in snakes.[11,14] Estimates of the prevalence of *Mycobacterium* in snake collections vary based on management practices from 0.1% to up to 30%.[11] Mycobacterial infections cause diffuse or multifocal inflammatory infiltrates which can appear as gray to white masses in the subcutaneous tissue and visceral organs, with the pulmonary system most commonly affected.[11] Acid fast staining, such as Ziehl-Neelson and Fite Farraco, can be used to identify the organisms,

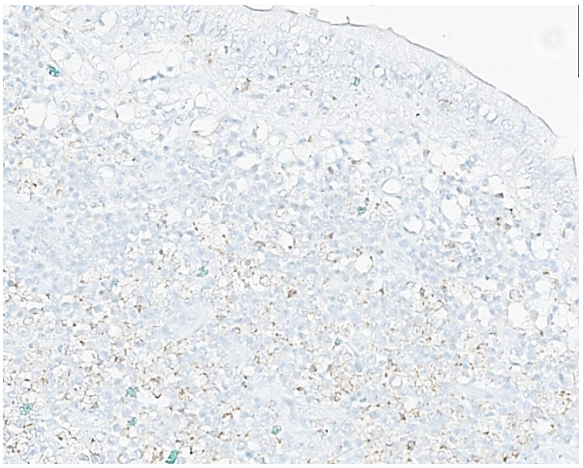

Figure 4-6. Few cells stain for IBA-1, including tingible body macrophages and infiltrating histiocytes. The neoplastic population is non-staining. (anti-IBA-1, 244X)

which may be rare or abundant, though occasionally the bacteria can be visualized on H&E or Gram staining.[14]

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