CASE I: JHU 63507 (JPC 4019896).

Signalment: Adult age unknown male eastern box turtle, (Terrapene carolina carolina).

History: A colony of 27 box turtles has been maintained at the Maryland Zoo in Baltimore for many years without major disease issues. In 2011, 2 of the turtles were found dead and considered to be too autolyzed for necropsy submission. At that time, clinical findings in many of the other turtles included lethargy, inappetence, plaques on the tongue, soft palate, and cloaca. Remaining turtles were triaged, separated based on severity of clinical signs, and treated with antimicrobials, gavage feeding, and additional supportive care. Some turtles were euthanized due to lethargy, severe oral and cloacal

1-1. Oral cavity, box turtle: The tongue, soft palate and hard palate are covered in multifocal to coalescing fibrinonecrotic plaques. (Photo courtesy of: The Department of Molecular and Comparative Pathobiology, Johns Hopkins University, School of Medicine, 733 N. Broadway St., Suite 811, Baltimore, MD 21205.)

1-2. Tongue, box turtle: The tongue is covered by a fibrinonecrotic membrane. (Photo courtesy of: The Department of Molecular and Comparative Pathobiology, Johns Hopkins University, School of Medicine, 733 N. Broadway St., Suite 811, Baltimore, MD 21205. http://www.hopkinsmedicine.org/mcp/index.html)
plaques, and overall clinical decline. Over six weeks a total of 13 box turtles died or were euthanized. This turtle was placed in the severely affected group when triaged and did not respond to supportive therapy. It was found dead three days after triage and was submitted to the Johns Hopkins Department of Molecular and Comparative Pathobiology for evaluation.

**Gross Pathology:** Gross findings included severe multifocal to coalescing fibrinonecrotic plaques within the oral cavity including the tongue and soft palate and the hard palate. Mucosal plaques into the proximal half of the esophagus and mild multifocal mucosal plaques were present in the cloaca. The stomach had intraluminal non-adherent fibrinonecrotic debris, and 1-6mm ulcerated mucosal nodules with adherent superficial fibrinonecrotic material. The turtle was in poor to fair body condition with scant perivisceral fat.

**Laboratory Results:** PCR positive for ranavirus; sequencing result in other individuals: *frog virus 3*, PCR negative for herpesvirus.

**Histopathologic Description:** Decalcified transverse section of the head, including oral cavity. The hard palate has multifocal to coalescing regions of mucosal loss with replacement by abundant fibrin, necrotic debris, and mixed heterophilic and lymphocytic infiltrates (including degenerate heterophils), with an overlying pseudomembranous crust consisting of sloughed epithelial cells, necrotic heterophils, fibrin, and bacterial colonies. At some edges of ulcerated areas, remnant squamous mucosa has ballooning degeneration, scattered intraepithelial heterophils and lymphocytes, intraepithelial edema, and occasional intracorneal foci of heterophilic and lymphocytic inflammatory cells. The submucosa contains multifocal perivascular lymphocytic and histiocytic infiltrates.

The lacrimal gland has multifocal mild interstitial lymphocytic inflammation with necrosis.

**Contributor’s Morphologic Diagnosis:** 1. Oral cavity (hard palate): Stomatitis, heterophilic and lymphohistiocytic, ulcerative, fibrinonecrotic, multifocal to coalescing, subacute, severe, with moderate submucosal lymphohistiocytic perivasculitis, superficial pseudomembrane formation, and superficial bacteria. 2. Lacrimal gland: Adenitis, lymphocytic, necrotizing, multifocal to coalescing, moderate.

**Contributor’s Comment:** The submitted case was one of 13 adult captive eastern box turtles from a zoological exhibit that all died over a span of several weeks. In addition to the severe stomatitis, microscopic findings included ulcerative and fibrinonecrotic glossitis, esophagitis, gastritis, cloacitis with pseudomembrane formation, and fibrinoid degeneration of vessel walls in the spleen,
subacute interstitial nephritis, subacute periportal hepatitis, lymphocytic enterocolitis, and lymphocytic perivasculitis in several organs. Inclusion bodies were not definitively identified in most cases and were rare in the lung and liver of one case. Incidental findings in several turtles included nodular gastritis with mixed necrotizing inflammation and intralysosomal nematode larvae. Primary differentials were ranavirus, herpesvirus, and septicemia.

Antemortem oropharyngeal samples were collected from many turtles and submitted for PCR detection. Ranavirus was confirmed in 8 of the 10 tested turtles submitted for necropsy, including all of the turtles with oral plaques similar to the submitted case. In two turtles, PCR was followed by DNA sequencing, identifying the ranavirus frog virus 3 in both cases. Herpesvirus was confirmed in 4 of the 10 tested turtles. Tissue from this turtle was negative for herpesvirus. Several bacterial agents were detected in oropharyngeal and blood samples from other ranavirus-positive turtles in this population, highlighting the potential role of secondary bacterial pathogens as factors contributing to inflammation, sepsis, and death of ranavirus-infected turtles.

Ranavirus currently is classified as a genus in the Iridoviridae family. Iridoviruses are large (120–200 nm), icosahedral, double stranded DNA viruses that replicate in the cytoplasm. Ranavirus infections are important causes of disease in fish⁵ and amphibians.⁴ The ranavirus frog virus 3 has been reported with increasing frequency as a significant cause of mortality in several reptile species.⁵ Environmental stressors, naïve or suppressed immunity, or introduction of novel strains may play a role in outbreaks that emerge in wild and captive reptiles. Amphibians and reptiles have been suggested as important reservoirs for ranaviruses that may cause economically and ecologically important disease in finfish.

Typical presentations of ranavirus infection in turtles includes cervical edema, palpebral edema, rhinitis, and stomatitis-glossitis.⁵ A series of cases of ranavirus in captive eastern box turtles in North Carolina¹ describes clinical signs that also included cutaneous abscesses, oral erosions and abscesses, and respiratory distress. Other studies that include several species of turtles and tortoises describe similar signs as well as yellow-white oral plaques.⁶,⁷ In these studies, histopathology revealed fibrinoid vasculitis of skin, mucous membranes, lungs, and liver, multifocal hepatic necrosis, multicentric fibrin thrombi, fibrinous

1-4. Oral cavity, hard palate, box turtle: There is full thickness necrosis of the mucosa overlying the hard palate, which is replaced with a serocellular crust. (HE 104X)
and necrotizing splenitis, and necrotizing stomatitis and esophagitis. While basophilic intracytoplasmic inclusion bodies have been reported in ranavirus infections, often they are not observed, even with ranavirus infection confirmed by PCR, electron microscopy, or virus isolation.


Conference Comment: Due to mild slide variation, the degree of lacrimal gland necrosis and inflammation within submitted sections varies; however, most conference participants appreciated some degree of necrotizing dacryoadenitis. The moderator concurred with this observation, but points out that reptiles and birds tend to have relatively high numbers of plasma cells within the normal lacrimal gland, so dacryoadenitis must be diagnosed with caution in these species. In addition to the differential diagnosis addressed by the contributor, including herpesvirus, bacterial septicemia and ranavirus, participants briefly discussed fungal infection (Candida spp.) and poxvirus as rule-outs for fibrinonecrotic stomatitis with pseudomembrane formation. These conditions can generally be differentiated histologically. Herpesvirus results in characteristic intranuclear viral inclusions, and poxvirus, while rarely reported in turtles, produces large intracytoplasmic inclusions. Candidiasis can be distinguished microscopically by the presence of budding yeast, pseudohyphae and true hyphae. In this case, viral inclusions were not identified and ranavirus was confirmed by PCR.

The contributor does an outstanding job of covering all the salient features of ranavirus infection in reptiles. Ranavirus, specifically frog virus 3, was initially associated with widespread disease epizootics in amphibians. Affected tadpoles (who are particularly vulnerable to infection) and frogs typically present with cutaneous hemorrhage/ulceration or disseminated disease with multiorgan necrosis. Subclinical infections are common in frogs; the kidneys and macrophage populations are considered the primary sites of virus persistence. Both adult and larval salamanders are susceptible to a ranavirus known as Ambystoma tigrinum virus, which results in splenic, hepatice, renal and gastrointestinal necrosis, sloughing of the skin, and discharge of inflammatory exudate from the vent. Interestingly, ambient temperature appears to play a significant role in disease pathogenesis, as high mortality is observed in those salamanders infected at 18°C, while those infected at 26°C tend to survive. Ranavirus infection in fish populations was first reported in Australian redfin perch and rainbow trout in the 1980’s; it has since been implicated in multiple disease episodes in both farmed and wild freshwater fish worldwide. Fingerlings and juveniles are most susceptible, and disease is characterized by severe necrosis in the liver, pancreas and renal/splenic hematopoietic cells. In addition to these tissues, Santee-Cooper virus, a ranavirus in wild largemouth bass, also causes enlargement and inflammation of the swim bladder, resulting in moribund fish that tend to float to the surface. As in amphibians, ranavirus infections in fish can be subclinical. Furthermore, inter-species transmission between amphibians and fish has been demonstrated, implicating both species as potential reservoirs for the virus. Ranavirus is such a significant problem in both fish and amphibians that it meets the criteria for listing by the World Organization for Animal Health (OIE).

Contributing Institution: The Department of Molecular and Comparative Pathobiology Johns Hopkins University, School of Medicine 733 N. Broadway St., Suite 811 Baltimore, MD 21205 http://www.hopkinsmedicine.org/mcp/index.html
References:
CASE II: R13/337 (JPC 4035417).

Signalment: Adult male garter snake, (*Thamnophis sirtalis parietalis*).

History: Recurring skin masses after surgical excision.

Gross Pathology: Located caudo-dorsally on the body there were two skin masses measuring 2 x 1 x 1 cm and 0.5 x 0.5 x 0.5 cm in size, covered by crusts. The masses were orange and firm and the larger mass infiltrated the underlying muscle. Located on the ventral side of the body there were two smaller nodules. The kidneys and liver were moderately enlarged and both organs showed multifocal to coalescing, poorly demarcated and infiltrative growing orange nodules of 0.1 x 0.1 x 0.1 cm up to 2 x 2 x 2 cm size. The coelomic cavity and hemipenis were also infiltrated by small multifocal orange masses.

Histopathologic Description: Liver: 90 - 95% of the tissue is replaced by a poorly demarcated, non encapsulated, infiltrative, multilobulated and densely cellular mass with small groups of remaining hepatocytes. Neoplastic cells are closely packed, supported by scant amount of fibrovascular stroma and arranged in anastomosing cords, islands and sheets. The neoplastic cells are polygonal to spindleoid, 15 to 30 µm in size with indistinct cell borders and have a high amount of granular eosinophilic cytoplasm and often contain birefringent olive-green to golden-brown granular pigment within the cytoplasm. Nuclei are round to oval to irregular with finely stippled chromatin and have up to three prominent nucleoli. Many cells contain up to five oval to irregular nuclei or have a huge nucleus (megakaryosis). Anisocytosis and anisokaryosis are high and there is anaplasia of neoplastic cells. In 10 HPF there are nine mitotic figures, some of which are bizarre. There are multifocal randomly distributed areas of necrosis, characterized by hypereosinophilia of the cytoplasm, pyknotic nuclei, karyolysis and karyorrhexis mixed with hemorrhage. Multifocally there are small groups and cords of remaining hepatocytes often showing hypereosinophilic, vacuolated and pyknotic nuclei (degeneration).

Contributor’s Morphologic Diagnosis: Liver: Iridophoroma, malignant, metastatic, garter snake (*Thamnophis sirtalis parietalis*), reptile.

Contributor’s Comment: Fish, amphibians and reptiles contain pigment cells in their skin that are called dermochromatophores.¹ These cells are derived from the neural crest and are characterized by the pigment they contain within their cytoplasm.¹ There are four types of dermochromatophores: melanophores, which contain melanin pigment, iridophores containing purines (colorless pigment), xanthophores containing carotenoids (yellow pigments) and erythrophores containing carotenoids and pteridines (red pigments).¹ Chromatophoromas are tumors arising from the cutaneous pigment cells that have been rarely reported²,³ and are subclassified into three types.

2-1. Skin, garter snake: Multiple orange-colored firm infiltrative nodules are present within the skin and extend into the underlying musculature. (Photo courtesy of: Institute of Animal Pathology, University of Berne, Länggassstrasse 122, Postfach 8466 CH-3001 Bern, Switzerland http://www.itpa.vetsuisse.unibe.ch/content/index_eng.html)
(melanophoromas or melanomas, xanthophoromas and iridophoromas) by the type of pigment they contain.\(^1\)\(^2\) Chromatophoromas with combined features of all cell types have also been described.\(^4\) The occurrence of these tumors in reptiles is higher than assumed and metastases are common.\(^1\)

Iridophoromas occur as intraepidermal white masses and metastases have been observed in several organs.\(^2\) Diagnosis of an iridophoroma is based on birefringent anisotropic granules within the neoplastic cells, distinguishing the iridophoroma from a melanophoroma and xanthophoroma. Based on the olive-green to golden-brown birefringent pigment within neoplastic cells we diagnosed a chromatophoroma of the subtype iridophoroma. Both benign and malignant iridophoromas have been reported in snakes, lizards, and fish.\(^1\)\(^3\) Malignancy criteria of chromatophoromas in reptiles encompass the presence of intravascular (lymphatic/blood vessel) neoplastic cells, visceral metastasis, high pleomorphism and the presence of mitotic figures.\(^2\)
Both Melan-A and S-100 protein can be detected by immunohistochemistry in iridophoromas.\(^2\)

**JPC Diagnosis:** Liver: Iridophoroma.

**Conference Comment:** The contributor provides an excellent summary of chromatophores and chromatophoromas in non-mammalian species. Chromatophores are contractile pigment cells that originate from embryonic neural crest cells and migrate to numerous tissues. Intracellular aggregation and dispersion of pigment granules engenders an ability to display rapid color changes in the skin, which can be important in camouflage, mating, and protection in many non-mammalian species.\(^3\) Based on the presence of birefringent material within neoplastic iridophores, conference participants on the whole concurred with the diagnosis of iridophoroma; however, there was also a lively debate concerning the histogenesis of a collection of morphologically distinct cells within the neoplasm. These cells appear slightly more individualized and polygonal, with atypical nuclear morphology and abundant dark-brown to occasionally greenish intracellular pigment that is not as birefringent. Although a consensus was not reached, discussion centered upon whether these cells represent a unique clonal variant of the more spindled neoplastic cell population, or perhaps, as some participants suggested, these are simply aggregates of melanomacrophages, which are a normal component of many reptile and amphibian livers, trapped within the neoplasm.

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**References:**
CASE III: 55819 (JPC 4032565).

Signalment: 1-month-old male Cape buffalo, *Syncerus caffer caffer*.

History: The calf was part of a small herd in a large field enclosure at a zoological park. It was noted to be depressed and slow for 5 days and was briefly caught for examination, collection of blood and fecal samples for diagnostic testing, and empirical treatment with a non-steroidal anti-inflammatory drug, antibiotics and an anthelmintic. The calf was found dead the next day.

Gross Pathology: The calf was 42 kg and had adequate adipose stores. In both kidneys, the renal papillae were streaked with pale yellow, finely granular material. Similar material was also present in variable amounts in calices and pelvices as sand-like to 2-3 mm diameter, rough, irregular, friable stones. The urinary bladder was distended with thin, cloudy, off-white urine.

Laboratory Results:

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Reference Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>3.7</td>
<td>2.8-3.8</td>
<td>g/dL</td>
</tr>
<tr>
<td>Total Protein</td>
<td>7.7</td>
<td>6.7-8.8</td>
<td>g/dL</td>
</tr>
<tr>
<td>Globulin</td>
<td>4.0</td>
<td>3.3-6.3</td>
<td>g/dL</td>
</tr>
<tr>
<td>BUN</td>
<td>148</td>
<td>10-23</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Creatinine</td>
<td>10.3</td>
<td>0.7-1.2</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>310</td>
<td>157-393</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Glucose</td>
<td>246</td>
<td>41-74</td>
<td>mg/dL</td>
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<tr>
<td>Calcium</td>
<td>6.7</td>
<td>9.1-11.3</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>14.8</td>
<td>5.1-8.7</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Chloride</td>
<td>132</td>
<td>93-100</td>
<td>mEq/L</td>
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<tr>
<td>Potassium</td>
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<td>4.1-5.5</td>
<td>mEq/L</td>
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<tr>
<td>Sodium</td>
<td>157</td>
<td>136-148</td>
<td>mEq/L</td>
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<td>Alk Phos</td>
<td>225</td>
<td>23-96</td>
<td>U/L</td>
</tr>
<tr>
<td>ALT</td>
<td>11</td>
<td>15-43</td>
<td>U/L</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>0.4</td>
<td>0.0-0.2</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

Kidney stone analysis (Minnesota Urolith Center): 100% xanthine

Histopathologic Description: Kidney: The calices are dilated and contain abundant irregular, rounded, lamellated, basophilic concretions (nephroliths), which are partially composed of and mixed with refractile, birefringent, brown to green crystalline material. These concretions and crystals are mixed with neutrophils and cellular debris and multifocally disrupt the renal papillae and urothelium of the calices, which is variably attenuated or hyperplastic. Throughout the section, tubules are frequently dilated and contain pale eosinophilic hyaline casts, coarse granular eosinophilic debris, sloughed necrotic epithelial cells, neutrophils, or lamellated concretions of amorphophilic, wispy, radiating, predominantly non-birefringent material similar to that in the calices. The tubular epithelium is often attenuated, necrotic or disrupted by the embedded concretions, which are sometimes covered with a layer of epithelium. There is multifocal mild fibrosis in the interstitium, and glomeruli are diffusely subjectively small.

Contributor’s Morphologic Diagnosis: Kidneys: Severe diffuse chronic nephrolithiasis with myriad intra-tubular and intra-calices calculi and crystals, tubular ectasia, hyaline and cellular casts, interstitial fibrosis, hypoplastic glomeruli and mild neutrophilic pyelonephritis.

Contributor’s Comment: This case is typical of xanthine urolithiasis, though this condition has not previously been reported in Cape buffalo. Small stones in the kidneys were composed of 100% xanthine by quantitative stone analysis. Accumulation of xanthine crystals and stones in renal tubules and calices results in obstruction, progressive tubular damage, and eventually renal failure. Xanthine urolithiasis is very rare in animals but has been described in Japanese Black calves, a Galician Blond beef calf, sheep, certain breeds of dogs (e.g. Cavalier King Charles Spaniels, Dachshunds), and cats. The disease in this buffalo calf was very similar to cases of xanthine nephrolithiasis in domestic cattle, in which calves present between 1 and 6 months of age with renal failure. The variably-sized, yellow to brown stones can be present in the kidneys, ureters, and bladder and are radiolucent. Diagnosis is based on stone analysis. In this case,
other supporting findings included myriad small, spiculate crystals on examination of urine sediment and failure of the renal concretions to stain with von Kossa and Alizarin Red in histologic sections.

Xanthine is a metabolite of purines normally converted to uric acid by xanthine oxidase. Xanthinuria and xanthine urolithiasis result from loss of function of this enzyme, which can be the result of a primary enzyme defect inherited as an autosomal recessive condition or from secondary inhibition of enzyme function. Cases of the secondary form are most often caused by treatment with allopurinol, an inhibitor of xanthine oxidase, and are thus most commonly seen in Dalmatian dogs treated for urate urolithiasis. Dietary deficiency of molybdenum, a component of an essential cofactor of xanthine oxidase, was thought to account for xanthine urolithiasis in sheep. The Cape buffalo calf in this report had not been treated with allopurinol and had normal levels of molybdenum on postmortem analysis of liver. It is therefore thought to have had a primary (hereditary) form of the disease, similar to cases in domestic cattle. A half-sibling of this calf (same sire, different dam) had previously died at 2 months of age with nearly identical lesions, although stone analysis was not performed in that case. Four prior calves from the same dam and sire were unaffected. Characterization of the hypothesized genetic defect in this group of Cape buffalo has not yet been attempted.

JPC Diagnosis: Kidney: Nephrolithiasis, intracalyceal and intratubular, moderate to severe, with mild suppurative pyelonephritis, tubular proteinosis and tubular necrosis.

Conference Comment: Urolithiasis, a condition frequently encountered in domestic animal species, refers to the presence of calculi within urinary passages. Calculi can form in any part of the urinary system from the kidneys to the urethra. Due to the length and narrow diameter of the penile urethra, impaction secondary to urethral calculi is common in males, especially at the ischial arch, the sigmoid flexure in ruminants, the vermiform appendage in rams, and the proximal end of the os penis in dogs. Calculi are grossly visible concretions of precipitated mineral, urinary proteins and debris, while urethral plugs are composed of aggregates of “sandy sludge” with a higher proportion of organic material that tend to conform to the shape of the urinary cavity that they fill. Urolith formation is influenced by familial, congenital and pathophysiologic factors including urinary pH, relative dehydration, infection, anatomic abnormalities, foreign bodies (such as grass awns) and drug administration. The supersaturation of urine with stone-forming salts is an important precursor to urolith nucleation. Crystal precipitation and aggregation also play a role in the development of urolithiasis, although crystalluria without calculus formation is a fairly common finding and the factors that promote/prevent the formation of uroliths are complicated and poorly understood. Horse urine,
for instance, is normally supersaturated with calcium carbonate and crystalluria is a normal finding on equine urinalysis, yet horses rarely develop urinary calculi.5

The important types of urinary calculi in various species are listed in table 1; it is also worthwhile to remember that many uroliths are of mixed composition (although one mineral may predominate). Silica calculi are a significant cause of urinary tract obstruction in pastured ruminants and are occasionally seen in male dogs, especially German shepherds and old English sheepdogs or dogs receiving a ration high in plant-derived ingredients (i.e. corn gluten or rice/soybean hulls). They are hard, white to dark brown, radiopaque and spherical/ovoid (in the urinary bladder) or angular/irregular (when in renal calyces) with a friable core.3

Miniature schnauzers appear predisposed, probably due to a familial susceptibility to urinary tract infections. Sterile struvite urolithiasis has been reported in a line of English cocker spaniels and beagles. The formation of struvite calculi within the urinary bladder of cats is one manifestation of an idiopathic condition known as feline lower urinary tract disease (FLUTD); Russian blues, Himalayans, Persians and castrated/spayed males and females are predisposed. Of note, struvite crystalluria is often seen in cats even without calculi; the reasons for this phenomenon are poorly understood. It should also be noted that amorphous urethral plugs containing Tamm-Horsfall mucoprotein, albumin, globulins, cellular debris and struvite crystals are a much more important cause of urethral obstruction in male cats, typically associated with concurrent urinary tract inflammation. Struvite calculi also occur in feedlot steers and sheep on a high grain ration. Much like cats, obstruction in

Struvite calculi are composed of magnesium ammonium phosphate hexahydrate; historically, they were inappropriately identified as “triple phosphate” calculi. Grossly, these radiopaque stones appear white to gray, chalky, smooth and easily fractured. They typically contain additional compounds such as calcium phosphate (which often forms a shell around the struvite), ammonium urate, oxalate or carbonate. Struvites are most important in dogs, cats and ruminants where they are commonly associated with infection. Ureases produced by bacteria such as Staphylococcus spp. or Proteus spp. increase the urine pH, which decreases struvite solubility, thus favoring the formation of calculi.

3-3. Kidney, Cape buffalo calf: Crystals are occasionally present within renal tubules. (HE 168X)
ruminants is typically due to formation of a gritty sludge, rather than discrete calculi, while a high phosphate diet predisposes sheep to struvite urolithiasis.\textsuperscript{3,5}

Oxalate calculi tend to develop as large, solitary, white to yellow bladder stones covered with sharp spines. They are composed of calcium oxalate monohydrate or calcium oxalate dihydrate, and although the pathogenesis is poorly understood, it likely involves some combination of hypercalcioria and hyperoxaluria. The major causes of hypercalcemia/hypercalciuria are briefly discussed in WSC 2013-2014, conference 13, case 3. Oxalic acid may be ingested in some foods or synthesized from glyoxylic and ascorbic acid. The formation of calcium oxalate uroliths is inhibited by dietary magnesium, which forms soluble complexes with oxalate, and citrate, which forms a similar complex with calcium. Oxalate uroliths are the second most common type of calculi encountered in dogs and have been associated with hyperparathyroidism, hypercalcemia, hyperadrenocorticism and exogenous steroid administration. Male miniature schnauzers, bichon frises, lhasa apsos, Yorkshire terriers, shih tzus and miniature poodles are predisposed. The prevalence of oxalate urolithiasis is increasing in cats and is likely related to dietary factors, although the specifics are not clear. Since oxalate is metabolized in the rumen, oxalate calculi are not generally considered important in ruminants; however, they are reported in sheep grazing grain stubble, although the source of the oxalates in these cases remains unknown.\textsuperscript{3,5}

Uric acid and urates are products of purine metabolism; calculi usually contain ammonium urate in combination with uric acid and phosphate. Uric acid/urate calculi are typically multiple, hard, laminated green-brown, radiodense spherical stones found within the urinary bladder. Urate stones are rarely reported in swine and cats but are most common in Dalmation dogs. In neonatal pigs in a negative energy balance, there is increased production of purine catabolites, which also predispose the formation of urate calculi. Urate urolithiasis in Dalmations results from an autosomal recessive defect in the hepatic uptake of uric acid, leading to its incomplete metabolism and accumulation. This defective transport system also inhibits tubular reabsorption of uric acid from the glomerular filtrate, resulting in urine that is supersaturated with urates.\textsuperscript{3,5}

Xanthine is another purine metabolite, which, as noted by the contributor, is normally degraded via xanthine oxidase to uric acid. Xanthine stones are irregular, yellow to brown-red, laminated, friable and radiolucent.\textsuperscript{3,5} This interesting case provides an excellent example of an uncommon condition (xanthine urolithiasis) in an unusual species. The contributor provides a thorough description of the gross pathology, histology, clinical-pathology and pathogenesis of xanthine urolithiasis in veterinary medicine.

Table 1. Composition of urinary calculi in select domestic animal species.\textsuperscript{3}

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Types</th>
<th>Uncommon Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ox</td>
<td>Silica</td>
<td>Xanthine</td>
</tr>
<tr>
<td></td>
<td>Struvite</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carbonate</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>Silica</td>
<td>Xanthine</td>
</tr>
<tr>
<td></td>
<td>Struvite</td>
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</tr>
<tr>
<td></td>
<td>Oxalate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>“Clover stones”</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carbonate</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>Struvite</td>
<td>Silica</td>
</tr>
<tr>
<td></td>
<td>Oxalate</td>
<td>Cystine</td>
</tr>
<tr>
<td></td>
<td>Purines (urate, uric acid, xanthine)</td>
<td>Calcium phosphate</td>
</tr>
<tr>
<td>Cat</td>
<td>Struvite</td>
<td>Urate</td>
</tr>
<tr>
<td></td>
<td>Oxalate</td>
<td>Cystine</td>
</tr>
<tr>
<td>Horse*</td>
<td>Carbonate</td>
<td>Urate (neonates)</td>
</tr>
<tr>
<td>Pig*</td>
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</tr>
</tbody>
</table>

* urinary calculi are uncommon in horses and rare in swine

**Contributing Institution:** Wildlife Disease Laboratories
Institute for Conservation Research, San Diego Zoo Global
http://www.sandiegozooglobal.org

**References:**
**CASE IV:** HE6491 (JPC 4033566).

**Signalment:** 2-year-old female green anaconda, (*Eunectes murinus*).

**History:** The snake was kept in a zoological garden and died suddenly.

**Gross Pathology:** The serosal surface of posterior half of small intestine was severely congested and the mucosal surface was diffusely covered with fibrinonecrotic exudate. The mucosa of large intestine had severe edema with mild fibrinous exudate on the surface.

**Histopathologic Description:** The normal structure of small intestine is largely lost but a portion of the lamina propria and tunica muscularis retains a normal lymphoid follicle. The lumen of small intestine is filled with fibrinonecrotic debris and the intestinal wall is diffusely and transmurally necrotic with moderate hemorrhage and edema. Smooth muscle fibers in the tunica muscularis are separated by edema and hemorrhage. Diffusely, macrophages and heterophils infiltrate the submucosa and extend to the serosal surface. Macrophages often contain hemosiderin and there are scattered multinucleated giant cells. Numerous amoebic trophozoites are admixed with multifocal aggregates of bacteria within the luminal necrotic debris. Amoebic trophozoites are also present within the submucosa and tunica muscularis; they are often vacuolated and rarely contain cellular debris. The trophozoites average 16 μm and range from 10 to 27 μm in diameter. They are positive for PAS stain. Cysts are also occasionally noted in and around the serosa. There is also multifocal vasculitis with thrombi and/or necrotic debris occluding the lumen. Serosal blood vessels are severely congested and occasionally contain amoebic trophozoites.

**Contributor’s Morphologic Diagnosis:** Small intestine: Enteritis, fibrinonecrotizing, transmural, acute, diffuse, severe, with numerous amoebic trophozoites.

**Contributor’s Comment:** Based on the species and histopathology as well as their size and morphology, amoebic trophozoites were tentatively identified as *Entamoeba sp.*, an obligate intracellular parasite with a direct life cycle. *E. invadens* is one of the most significant protozoal parasites of reptiles, especially captive snakes and lizards. *E. invadens* trophozoites range from 10 to 35 μm in diameter and the cysts range from 10 to 20 μm, with up to four nuclei. Although the trophozoites and quadrinucleated cysts described above are morphologically indistinguishable from the primate amoeba, *E. histolytica*, *E. invadens* prefers a host or culture temperature of less than 31°C whereas *E. histolytica* evolves to thrive at 37°C.

Herbivorous turtles are thought to serve as the

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4-1. Ileum, green anaconda: The ileal mucosa is diffusely and circumferentially necrotic. (Photo courtesy of: Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan http://www.hokudai.ac.jp/veteri)
natural host for *E. invadens*. In turtles, the parasite likely lives as a commensal symbiont without any pathogenicity. While in the trophozoite state, these protozoa locomote and feed by forming pseudopodia. The resistant cyst stage is excreted in the feces. Following contamination of the water supply of snakes and lizards, the protozoa are ingested and induce a fulminating enteritis and hepatitis in these species. The clinical signs include regurgitation of undigested food and severe diarrhea, occasionally accompanied by blood- or bile-tinged green mucus, and/or remnants of intestinal mucosa.

The most characteristic microscopic lesion induced by *E. invadens* is severe intestinal erosion, ulceration and inflammation, often with formation of fibrinonecrotic pseudomembranes (diphtheritic enteritis). Typically, the ileum and colon are the most severely affected intestinal segments and the affected gut wall is severely thickened. The protozoa invade blood vessels, spread systemically and induce necrosis and inflammation in various extra-intestinal tissues, especially the liver.

This anaconda has typical diphtheritic ileitis with numerous amoebic trophozoites and similar (though less severe) inflammation is observed in the large intestine. Although the protozoa often migrate in the sinusoids of liver and occasionally in the vessels of lung and kidney, there is no apparent damage to these organs in this case. We conferred with a veterinarian in the zoological garden in an attempt to elucidate the mechanism of infection in this case and were unable to reach a definitive conclusion; however, we suspect that the anaconda’s water supply was contaminated by turtle feces.

Immunosuppression has been suggested as an important predisposing factor in snake *Entamoeba* infections. There is a recent report of diphtheritic colitis caused by an amphibian *Entamoeba* sp. in a Boa constrictor kept in a zoological garden. Histopathological changes in this case were limited to the large intestine, implying that this *Entamoeba* may have a lower pathogenicity than *E. invadens*. As the snake suffered simultaneously from endoparasitism and inclusion body disease, the animal was suspected to be in an immunocompromised state. Our case also demonstrated moderate atrophy of lymphoid follicles in spleen, which may be a result of inadequate nutrition; however, the details are unclear.
JPC Diagnosis: 1. Intestine: Enteritis, fibrinonecrotic, circumferential, diffuse, severe, with numerous amoebic trophozoites.  

Conference Comment: Entamoeba spp. is a protozoan parasite belonging to the phylum Sarcomastigophora, subphylum Sarcodina (Rhizopoda), order Amoebida, family Entamoebidae, genus Entamoeba.3 Amoebae are normal, nonpathogenic inhabitants of the large bowel lumen in many species. Disease occurs upon invasion of the intestinal mucosa, and severity is influenced by diet, immune status, and the particular strain and virulence of the organism.8 E. invadens and E. histolytica are two commonly important species in veterinary medicine, both of these amoebae have similar life cycles with two morphologically distinct stages. The labile trophozoite inhabits the host and is capable of locomotion, while the resistant quadrinucleate cyst form is protected by a cell wall and is able to survive under unfavorable environmental conditions. The cyst is infectious, and releases the motile trophozoite following ingestion by the host.1,3 Virulence factors of Entamoeba spp. include 1) a Gal/GalNAc-specific lectin, which facilitates trophozoite adhesion to intestinal epithelial cells and may contribute to amoebic resistance to complement, 2) pore-forming polypeptides called amoebapores that insert a channel into the host cell membrane, leading to cell lysis, and 3) a family of cysteine proteases that function to break down the extracellular matrix, allowing amoebic tissue invasion.1,5

The contributor provides an excellent summary of Entamoeba invadens in reptiles. Chelonians and crocodilians are considered to be the natural hosts of E. invadens and may serve as a reservoir for infection in snakes and lizards in captivity. As noted by the contributor, transmission is typically feco-oral (probably related to contamination from turtle feces) and the most common presentation of invasive amoebiasis in reptiles is enteritis, often with subsequent hepatitis following hematogenous dissemination via the portal vein. After entering the mesenteric circulation, trophozoites may disseminate to other organs, although this is uncommon. There is a single report of amoebic myositis in a water monitor (Varanus salvator), presumably due to either hematogenous spread or direct invasion via skin wounds.1

Entamoeba histolytica is the etiologic agent of amoebic dysentery in humans, with an incidence of up to 500 million clinical cases per year,
including up to 100,000 fatalities. E. histolytica is distributed worldwide among humans, but also occurs in a broad range of New and Old World monkeys and apes, and can be transmitted to dogs, cats, cattle and macropods; the pathogenesis is similar to E. invadens. In Old World monkeys, amoebic dysentery typically induces necrotizing colitis, with occasional dissemination to the liver or (rarely) other tissues. Conversely, in certain species of leaf-eating monkeys, including colobus monkeys, silver leaf monkeys, douc langurs and proboscis monkeys, fibrinonecrotizing gastritis is the principle lesion associated with E. histolytica. It is thought that the normal neutral pH within these gastric compartments provides a favorable environment for excystation of ingested E. histolytica, followed by tissue invasion. E. histolytica can be transmitted to dogs, cats and cattle, where it typically causes colitis; gastric amoebiasis has been reported in a wallaby, a species with a complex, sacculated stomach adapted for fermentation that is similar to that of leaf-eating monkeys.

In addition to severe fibrinonecrotic enteritis, conference participants found the multifocal necrotizing vasculitis and thrombosis within adjacent mesenteric vessels particularly striking. The etiology of this finding is unclear; discussion centered upon the possibility of direct damage from amoebic trophozoites versus the potential of a concomitant gram-negative septicemia (i.e. salmonellosis) with subsequent vasculitis.

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References: