CASE I - M03-1654 (AFIP 2886856)

Signalment: Four-year-old male corn snake (Elaphe guttata guttata).

History: This animal belonged to a collection of seventy-five snakes. Thirty-eight snakes were experiencing weight loss and regurgitation and some had died. This snake was euthanized due to chronic weight loss, regurgitation and diarrhea.

Gross Pathology: The referring veterinarian noted a thickened gastric mucosa at necropsy. Tissues from this snake were fixed in formalin and mailed to the pathology department.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Diffuse gastric mucosal hyperplasia with intra-lesion Cryptosporidium and bacteria.

Contributor's Comment: The gastric mucosa is markedly hyperplastic with prominent mucosal folds lined by multiple layers of columnar epithelium with basal nuclei. The chief cells are completely replaced by mucous neck and surface epithelial cells. There are nests of hyperplastic branching glands extending deep into the lamina propria. Focal infiltrates of lymphocytes, plasma cells, and a few granulocytes are scattered throughout the lamina propria. Small 4-5um oocysts are present in a few gastric glands and are attached to the luminal surface of epithelial cells. Surface epithelium and gastric pits are overgrown with mixed populations of gram-negative and gram-positive bacterial cocci and rods.

Coccidia of the genus Cryptosporidium infect mammals, birds, fish, and reptiles. Transmission is direct by sporulated or unsporulated oocysts shed in the feces.
Cryptosporidiosis is a well-known cause of gastric hyperplasia (hypertrophic gastritis) in captive snakes. In the present case, there was overgrowth of bacteria on the surface and in gastric pits, possibly secondary to gastric hyperplasia. Chronic postprandial regurgitation and loss of body mass are the most common presentations in infected snakes. The disease is often protracted and fatal\(^1\). As in this case, outbreaks of cryptosporidiosis can devastate ophidian collections. Snakes shed large numbers of oocysts in the feces and the disease can be extremely difficult to eradicate from a collection\(^1,2\). Stringent sanitation and quarantine procedures are required to contain the disease. Hydrogen peroxide and formalin are effective disinfectants\(^1\). The classification and speciation of *Cryptosporidium* is still uncertain\(^3\). *Cryptosporidium serpentis* is the pathogenic species for snakes and other reptiles. The reptilian pathogen appears not to infect mammalian and avian species and mammalian and avian species are non-infectious for snakes. However, snakes can be infected with *C. serpentis* from other species of reptiles\(^2\).

**AFIP Diagnosis:** Stomach: Mucosal neck cell hyperplasia, diffuse, moderate, with granular cell loss, submucosal edema, and apical protozoal organisms, etiology consistent with *Cryptosporidium* sp., corn snake (*Elaphe guttata guttata*), reptile.

**Conference Comment:** This case was reviewed in consultation with the Department of Pathology, National Zoological Park. Conference attendees noted the high number of bacteria within the gastric pits and on the surface epithelium and concluded that it is most likely overgrowth due to retention of ingesta and slowed digestion secondary to the *Cryptosporidium* sp.

*Cryptosporidium* sp. are located at the apical surface of epithelial cells. Ultrastructurally, the organism resides in an intracellular but extracytoplasmic environment. The organism is surrounded by a host cell-derived membrane (parasitophorous vacuole) and attaches to the epithelial cell by a specialized feeder organelle, displacing microvilli. While *Cryptosporidium serpentis* is the most common species in reptiles, *Cryptosporidium parvum* is most common in ruminants, and *Cryptosporidium baileyi* in poultry. Heavy infections are reported in immunocompromised animals, such as chickens with infectious bursal disease (birnavirus) or cats with feline leukemia virus infection (retrovirus)\(^4,6\).

Proliferative gastritis is a characteristic finding in snakes infected with *Cryptosporidium* sp. Causes of proliferative gastritis/abomasitis in other species include *Ostertagia ostertagi* in cattle, *Ostertagia circumcincta* in sheep and goats, *Nochtia nocti* in nonhuman primates, *Trichostrongylus axei* in horses, *Hyostrongylus rubidus* in pigs, and *Ollulanus tricuspis* in cats\(^4,5\).

Histologically, the gastric mucosa of reptiles lacks the parietal cells present within the mammalian stomach. In reptiles, the gastric glands are composed solely of chief and clear cells\(^7\).
CASE II - 2 (AFIP 2891621)

Signalment: Captive-bred, adult of unknown age, male yellow napped amazon parrot (Amazona auropalliata).

History: Sudden death.

Gross Pathology: Pale, mottled, hemorrhagic, enlarged liver. Hemorrhages in multiple tissues, particularly the mesenteric fat and liver.

Laboratory Results: Positive for psittacid herpesvirus (Pacheco’s Disease Virus) by in-situ PCR.

Contributor’s Morphologic Diagnosis: Massive, diffuse, acute hepatocellular necrosis with syncytial cell formation, intranuclear inclusion bodies, and multifocal hemorrhage (Pacheco’s disease).

Contributor’s Comment: Pacheco’s disease (PD) is caused by a heterogeneous group of psittacid herpesviruses (PsHVs) closely related to Gallid herpesvirus-1.1-3
Since its initial description in Brazil in 1929, Pacheco’s disease has attained worldwide distribution with many reported outbreaks in the United States. Recent investigations in Europe and the United States indicate that there are at least 5 serologic serotypes and 10 genetic variants, and that infection by one serotype/variant may not be protective against infection by other variants. In spite of the heterogeneity, some variants occur more commonly than others. For example, in one study the majority (63%) of reported cases were caused by a single genetic variant.

High morbidity and mortality are typically seen in affected flocks, particularly those with poorly managed, densely populated aviaries; however, individual pet birds may also be affected. Sudden death or minimal, brief, non-specific clinical signs characterize PD, and infected birds may die 3-14 days after exposure. Latently infected birds are thought to be a source of infection and environmental contamination. The bird in this case was from a single-bird household with no recent contact with other birds. We speculate that the bird may have been latently infected and suffered a relapse of the disease or the owner may have brought the infection to the household from a contaminated source.

Macroscopic lesions that may be associated with PD consist of hepatomegaly, splenomegaly, pale discoloration of the liver and spleen (necrosis), and hemorrhage in multiple tissues. Less frequently, macroscopic lesions may be identified in other organs. Microscopically there is multifocal to massive, peracute to acute hepatocellular necrosis without significant inflammatory infiltrates. Necrosis may be so severe and extensive that the hepatic lobular architecture is completely distorted as in the present case (Fig. 1). Intranuclear inclusion bodies are commonly seen in hepatocytes and biliary epithelium. Syncytial cells may be identified in hepatic tissue but are reportedly not common. Splenic necrosis with intranuclear inclusion bodies are a common finding. Other tissues are affected less frequently and may contain intranuclear inclusion bodies (gastrointestinal tract, pancreas, lymphoid tissues, bronchi, kidneys, ovary, thyroids/parathyroids). The present case was characterized by hepatic and splenic necrosis with hemorrhage in multiple tissues (Figs. 1 and 2). Hepatic syncytial cells were infrequently seen (Fig. 3), inflammatory infiltrates (heterophils) were minimal (Fig. 1), and intranuclear inclusion bodies were present in hepatocytes (Fig. 4), biliary epithelial cells (Fig. 3), and mononuclear cells in the spleen (Fig 2).

The macroscopic and microscopic lesions are not specific for PD/PsHVs since similar lesions may be seen with other viruses (avian polyomavirus, avian adenovirus). Other causes of hepatic necrosis include bacterial infections (Chlamydophila psittaci, Salmonella sp., Clostridium piliforme), viruses (avian reovirus, psittacine circovirus), trematodes, and toxins (aflatoxins). A specific diagnosis is possible with in-situ PCR and whole tissue PCR assays, fluorescent antibody testing, and immunohistochemistry. In the present case the histologic findings were strongly suggestive of PD and in-situ PCR was diagnostic for PsHVs. The primer probes utilized in this assay are based on a conserved nucleotide sequence present in multiple variants of PsHV (K. Latimer, personal communication).
Captions for figures:
Fig. 1. Massive and diffuse hepatocellular necrosis with minimal heterophilic infiltrates. (H&E, 60X)
Fig. 2. Splenic necrosis with intranuclear inclusion bodies (arrows). (H&E, 60X)
Fig. 3. Numerous biliary epithelial cells with intranuclear inclusion bodies and syncytial cell formation (arrows). (H&E, 60X)
Fig. 4. Hepatocellular (arrows) and biliary (arrowhead) intranuclear inclusion bodies (arrows). (H&E, 60X)

AFIP Diagnosis: Liver: Hepatocellular necrosis and degeneration, diffuse, severe, with rare syncytia and many eosinophilic intranuclear inclusion bodies, yellow napped amazon parrot (Amazona auropalliata), avian.

Conference Comment: The contributor gives a concise review of Pacheco’s disease and important differential diagnoses. In addition to Pacheco’s disease virus (psittacid herpesvirus-1), other alphaherpesviruses of birds include gallid herpesvirus-1 (avian infectious laryngotracheitis), gallid herpesvirus-2 (Marek’s disease), and anatid herpesvirus-1 (duck plague). Significant gross lesions associated with infectious laryngotracheitis include thickened and hemorrhagic tracheal mucosa with necrotic debris in the tracheal lumen. Marek’s disease virus causes atypical lymphocyte proliferation in a variety of tissues, including peripheral nerves, bursa, thymus, iris, and visceral organs. Duck plague, or duck viral enteritis, causes hemorrhage and necrosis in the gastrointestinal tract, liver, and lymphoid organs, to include the gastrointestinal associated lymphoid tissue (GALT), which produces the characteristic dark annular bands in the intestinal mucosa.4

The contributor provided a list of differential diagnosis for hepatocellular necrosis in psittacines, most notably Chlamydophila psittaci, adenovirus, and polyomavirus. Clusters of Chlamydophila psittaci organisms within hepatocytes and macrophages are diagnostic for psittacosis but may require special stains such as Gimenez. Polyomavirus and adenovirus cause large intranuclear amphophilic to basophilic inclusions that expand the nucleus.3

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References:

CASE III - 44678 (AFIP 2890230)

Signalment: 2 year 8 month-old, female, frilled lizard, Chlamydosaurus kingii, reptile.

History: This lizard was hatched at the San Diego Zoo on 7 November 1999. It was presented on 18 July 2002 for weight loss and swelling of the right carpus. On physical examination the animal was emaciated and had a poor righting reflex. Euthanasia was elected.

Gross Pathology: At necropsy the right forelimb was mildly swollen from the elbow to the phalanges. The limb measured 1.0 cm diameter at its widest point. All other organs examined were grossly normal.

Laboratory Results: None reported.

Contributor’s Morphologic Diagnoses:
1. Severe regionally extensive articular and periarticular deposition of amphophilic material with granulomatous inflammation and bone remodeling (Severe articular and periarticular gout).
2. Moderate focal subcutaneous deposition of amphophilic material with granulomatous inflammation (Moderate subcutaneous gout).
Contributor’s Comment: The section examined is through the radius and carpus. It includes the articular surfaces of the carpal bones, adjacent skeletal muscle and a section of the overlying skin. Lesions vary in severity and distribution from slide to slide. Surrounding the carpal joint and dissecting between the muscle fascicles are large aggregates of amphophilic, finely granular material. These aggregates are surrounded by a thin fibrous connective tissue capsule and mildly compress the adjacent skeletal muscle fibers. Thin fibrous connective tissue trabeculae often extend from the capsule, further subdividing the amphophilic material. Small deposits of amphophilic material within the skeletal muscle demonstrate radiating thin crystals, classic for gout tophi. A similar aggregate of material is present between the skin and the underlying skeletal muscle. Amphophilic granular material and scanty inflammatory cell aggregates are present within the carpal joint spaces. In some areas near the capsule the material is birefringent.

The fibrous connective tissue capsule is lined by an inner rim of inflammatory cells. The inflammatory cells are predominantly epithelioid macrophages with frequent multinucleated giant cells, few lymphocytes and plasma cells. Similar inflammatory cells are intermingled with the amphophilic granular material in the joint spaces. The articular cartilage surfaces are irregular and mildly fibrillated. Along one margin of the radius there is proliferation of woven bone beneath the periosteum associated with a small aggregate of amphophilic material.

Gout is a relatively common disease of certain captive and wild reptiles. It is also a disease of humans, non-human primates, birds and canids, particularly the Dalmatian dog. The disease is caused by either increased production of uric acid (humans, Dalmatian dog) or a disruption in the balance between uric acid production and excretion (reptiles, birds). The most common causes of disruption in reptiles are a high protein diet, (eg., herbivorous reptiles fed a carnivorous diet), and chronic disease, especially renal tubular disease and dehydration. Administration of specific drugs (eg., furosemide, aminoglycosides, sulfonamides) can also result in decreased excretion of uric acid either through retention of urates (furosemide) or renal tubular damage (aminoglycosides, sulfonamides).1,2

Uric acid is an end product of degradation of dietary protein in humans, primates, the Dalmatian dog, birds and certain reptiles (terrestrial chelonians, all lizards and snakes).3 Specifically, uric acid is the end product of purine nucleotide breakdown. Briefly, dietary protein is degraded into individual nucleic acids. These nucleic acids are broken down by nucleases to individual nucleotides, which are further hydrolysed into individual purine and pyrimidine bases. Pyrimidines are catabolized into CO2 and NH3. Purines undergo further degradation by xanthine oxidase to uric acid.1,2

In reptiles uric acid is cleared from the blood via the renal tubules. This is different from most mammals where clearance is accomplished through glomerular filtration. In blood, uric acid is present both as free uric acid and urate salts, both of which are relatively insoluble in water. When the concentration of either of these forms becomes
elevated in the blood (hyperuricemia) or body fluids (e.g., synovial fluid) the uric acid and salts crystallize forming insoluble precipitates deposited in tissues throughout the body.\textsuperscript{1-3}

These precipitates or “gout tophi” are frequently grossly visible at necropsy. In reptiles the most common sites of deposition are the pericardium, kidneys, liver, spleen, lungs, subcutis and other soft tissues.\textsuperscript{1,4} A definitive diagnosis of gout is made by demonstrating monosodium urate crystals within the joints or affected tissues. There are diseases that result in the deposition of crystals other than sodium urate, which result in similar gross and histologic findings. This condition is referred to as pseudogout.\textsuperscript{1,2}

In this case the animal was being fed an appropriate diet and had adequate water available. Histologic examination of the kidneys revealed possible moderate interstitial fibrosis (and euthanasia solution artifact). Within the oviduct there was a severe focal subacute ulcerative salpingitis with intralesional gram-positive bacteria. Other organs demonstrated multifocal acute vascular fibrinoid necrosis. These findings suggest that this animal may have been septic prior to euthanasia. It is possible a combination of decreased renal function and decreased water intake due to underlying sepsis resulted in the development of articular gout in this case.

\begin{quote}
**AFIP Diagnosis:** Carpus and associated soft tissue: Arthritis, tenosynovitis, and myositis, granulomatous, multifocal to coalescing, severe, with reactive bone and numerous urate tophi (gout), frilled lizard (\textit{Chlamydosaurus kingii}), reptile.
\end{quote}

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**Conference Comment:** The contributor gives a concise review of pathologic mineralization. There are two forms of gout in birds and reptiles: visceral and articular. The visceral form is more common and presents grossly as white or gray chalky patches on the pericardium, liver, mesentery, and peritoneum, and renal interstitial or subcapsular deposits. The articular form is much less common and is characterized by swollen joints with white deposits on tendon sheaths.\textsuperscript{5}

Tophi are crystalline structures with spicules that radiate from the center in a "starburst" fashion and can be stained with Gomori’s methenamine silver (GMS).\textsuperscript{6} Since urates are water soluble, urate deposits are leached when tissues are formalin-fixed. It may be preferable to collect tissues in absolute ethyl alcohol for best visualization of tophi.\textsuperscript{3}
\end{quote}

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**References:**
CASE IV - N2003-72 (AFIP 2897595)

Signalment: Approximately 6 1/2 year old, male, cloud rat (*Phloeomys pallidus*).

History: This cloud rat was thin and dyspneic. Pneumonia was confirmed on radiographs and the animal was placed in an oxygen cage and started on a course of intraosseous antibiotics and antifungals and antimicrobial nebulization. The rat died 5 days later.

Gross Pathology: The cloud rat was in thin body condition. Multiple fibrous adhesions were present between the pleura and the thoracic wall, pericardium, and diaphragm. The lungs had an irregular contour and approximately 90% of the parenchyma was firm and diffusely pale tan-gray with multifocal red mottling. On section, the lungs were firm and tan-white with multiple pockets and cavitations containing thick, yellow-white, often pasty material. The tracheobronchial/bronchial lymph nodes were enlarged, firm, and diffusely light tan with poor distinction between cortical and medullary tissue.

Laboratory Results:
- Antemortem: positive *Cryptococcus* titer using latex agglutination
- Post-mortem:
  - *lung cytology*: numerous organisms consistent with *Cryptococcus neoformans*
  - *lung fungal culture*: *Cryptococcus neoformans* (biotyping of variant not performed)

Contributor's Morphologic Diagnosis: Lung: Pneumonia, necrotizing, fibrosing, histiocytic to granulomatous, chronic, diffuse, severe with myriad intralesional fungal organisms consistent with *Cryptococcus neoformans*. 
**Contributor's Comment:** *Cryptococcus neoformans* is a saprophytic heterobasidiomycetous yeast that appears in tissue sections as uninucleate, thin-walled, spherical, oval and elliptical cells that vary in size from 3.5 to 8um or more in diameter.\(^1,^2\) The yeast are surrounded by a mucopolysaccharide capsule that are unstained to very lightly stained with hematoxylin and eosin staining, and vary in appearance from wide spherical halos (“soap bubble” appearance) to nearly undetectable lighter zones around the cells.\(^1,^2\) The capsular material is usually readily demonstrated with mucin stains (Alcian blue, Mayer’s mucicarmine) and PAS staining. In tissues, *Cryptococcus* species reproduce asexually as blastoconidia with narrow-based, most often single, budding.\(^2,^3\) Chains of budding cells may be observed; pseudohyphae and branched, septate hyphae are rarely produced in tissues.\(^1,^2\) The host response to infection with *C. neoformans* usually depends on the immunologic status of the host, the presence of underlying disease and whether or not the cryptococci are encapsulated.\(^2\) Inflammation can vary widely from none to an intense, supplicative and necrotizing reaction with subsequent granuloma formation and eventually fibrocaceous lesions. The relatively nonantigenic polysaccharide capsule inhibits plasma cell function, macrophage phagocytosis and leukocyte migration.\(^4\) In older fibrocaceous lesions, GMS staining may be needed to demonstrate organisms. It is often difficult to isolate fungus from these lesions; a presumptive histologic diagnosis can be confirmed with immunohistochemistry.

There are two recognized biovariants of *Cryptococcus neoformans* that cause disease in both humans and animals. *Cryptococcus neoformans var. neoformans* has nearly worldwide distribution. It is most frequently associated with bird droppings (the organism utilizes creatine in the droppings), especially those of pigeons, and soils contaminated with bird manure.\(^1\) *Cryptococcus neoformans var. gatti* occurs mainly in tropical, subtropical, and temperate climates and in association with certain species of gum trees (*Eucalyptus camaldulensis* and *E. tereticornis*).\(^5\) In humans, *C. neoformans var. neoformans* more often affects and causes more severe disease in immunocompromised individuals, whereas *C. neoformans var. gatti* is repeatedly isolated primarily from immunocompetent hosts.\(^5\)

Pulmonary and cerebromeningeal cryptococcosis are the two main forms of disease in humans and several species of domestic and wild animals.\(^2,^4,^6\) Infection occurs by inhalation of aerosolized fungal cells from the environment.\(^2\) The clinical course of pulmonary cryptococcosis is subacute or chronic and frequently is complicated by concomitant extrapulmonary infection. Additionally, about 1% of human patients with first-infection cryptococcosis develop a primary pulmonary-lymph node complex.\(^2\) *Cryptococcus neoformans* has neurotropism and frequently spreads to the central nervous system from the respiratory tract either hematogenously\(^2\) or via direct extension through the cribriform plate.\(^3,^4\) Other sites are involved in disseminated infection and cutaneous lesions (primary or secondary) can occur in humans and animals.

Cryptococcosis is the most common systemic fungal disease of domestic cats.\(^3,^4\) It has been reported in 2 cheetahs.\(^4\) Some studies have noted an increased incidence of FeLV or FIV in cases of feline cryptococcosis.\(^3\) However, concurrent underlying
diseases are often not detected in domestic and exotic felids with cryptococcosis.\textsuperscript{3,4} Therefore, though immunosuppression has been suggested as a predisposing factor in cryptococcal infection, it is difficult to make valid conclusions about the relationship between cryptococcosis and immunosuppression in cats.\textsuperscript{4} In dogs, nasal cryptococcosis is the primary form, although disseminated systemic infection occurs. An association between cryptococcal infection and immunosuppression has not been documented in dogs.

There are sporadic reports of cryptococcosis in captive nonhuman primates, especially in Old World species. Reports in New World monkeys include a squirrel monkey (pulmonary and lymph nodes),\textsuperscript{2} Geoffrey’s tamarin (disseminated disease) and a common marmoset with a 1 month history of wasting (intestinal and mesenteric lymphatics).\textsuperscript{7} Intestinal involvement is not common in animals or humans but it has been sporadically identified as a opportunistic enteric pathogen in AIDS patients with diarrhea.\textsuperscript{7}

Respiratory and central nervous system infection has been reported in several groups of captive bred and wild-caught, captive elephants and tree shrews.\textsuperscript{6} Environmental exposure to the organism may be enhanced by exhibit conditions and/or behavioral factors, and there is evidence to suggest that tree shrews may have a predilection for cryptococcosis.\textsuperscript{6}

Cloud rats (\textit{Phloeomys pallidus}) are large, nocturnal, arboreal Asian rats. Necropsy results show that six of eighteen adult (>1 year of age) captive cloud rats (including the case submission) housed at the same facility but in three different locations have died between 1989 and 2003 due to cryptococcal infection. Affected animals were between 4 and 9 years of age. The respiratory tract was the most commonly affected organ system. Some animals died without premonitory signs. The cloud rat exhibits contain trees and other substrates that might be suitable sites for proliferation of \textit{C. neoformans}, and it is suspected that the rats were exposed to the fungus via contaminated substrate. The immunologic status of the affected animals was unknown and significant concurrent disease was not always present. Further investigation including immunologic studies would be needed to determine whether the cloud rat is a species that is particularly susceptible to cryptococcosis.

\textbf{AFIP Diagnosis:} Lung: Pneumonia, necrotizing, chronic, diffuse, severe, with myriad yeast, etiology consistent with \textit{Cryptococcus neoformans}, cloud rat (\textit{Phloeomys pallidus}), rodent.

\textbf{Conference Comment:} This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant for veterinary parasitology. The contributor gives a thorough review of cryptococcosis. Conference attendees noted there was variation in the degree of fibrosis present among slides.
Differential diagnoses for fungal infections that cause granulomatous pneumonia include *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum*. *Blastomyces*, *Coccidioides*, and *Histoplasma* are unencapsulated, unlike *Cryptococcus*. *Blastomyces* reproduces by broad-based budding, while *Cryptococcus* and *Histoplasma* reproduce by narrow-based budding. *Coccidioides* reproduces by endosporulation. Mature sporangia of *Coccidioides* are 10-80μm in diameter with a double-contoured wall and are filled with 2-5μm diameter endospores. *Histoplasma* is much smaller (2-4um diameter) than *Cryptococcus* and is located intracellularly within macrophages.8,9

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**References:**
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