Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2011-2012

Conference 8

02 November 2011

CASE I: 1 (JPC 4002931).

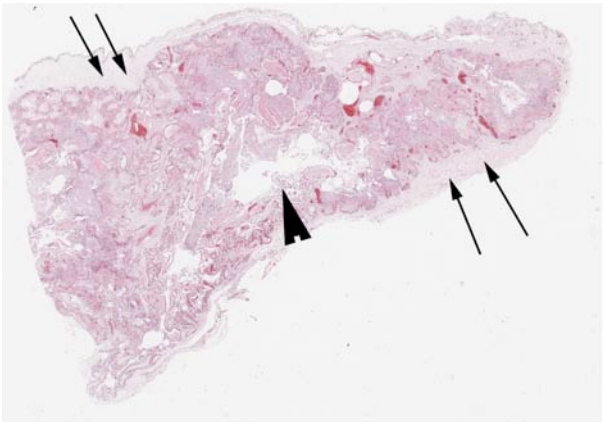
Signalment: Greek tortoise male 6 years *Testudo graeca*.

History: This was a pet tortoise that awoke from hibernation with nasal and ocular discharge. At clinical examination the tortoise was dehydrated and had a severe intestinal parasitic infection (ascarids and oxyurids) diagnosed by the referring veterinarian. Despite antibiotic, antiparasite and rehydrating therapy, clinical signs including open mouthed breathing worsened and the tortoise died spontaneously.

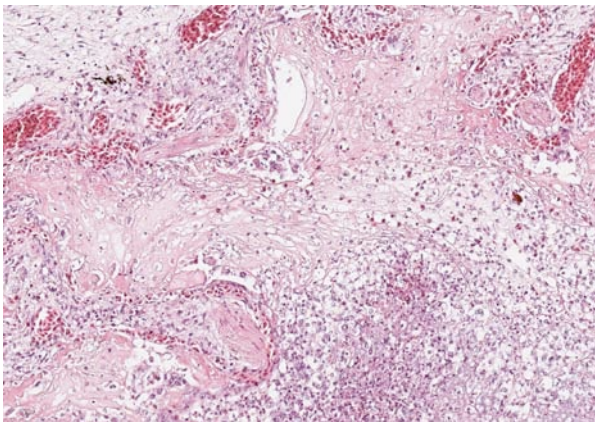
Gross Pathology: A full necropsy was performed by the referring veterinarian who reported the presence of ulcerative glossitis and stomatitis with pannus formation and severe hyperaemia of lungs. Esophageal and tracheal mucosal linings were reported to be diffusely ulcerated. Heart, liver, kidney, spleen, testes, stomach and intestine were grossly normal.

Several organs, including the lungs, were formalin-fixed and sent for histopathology.

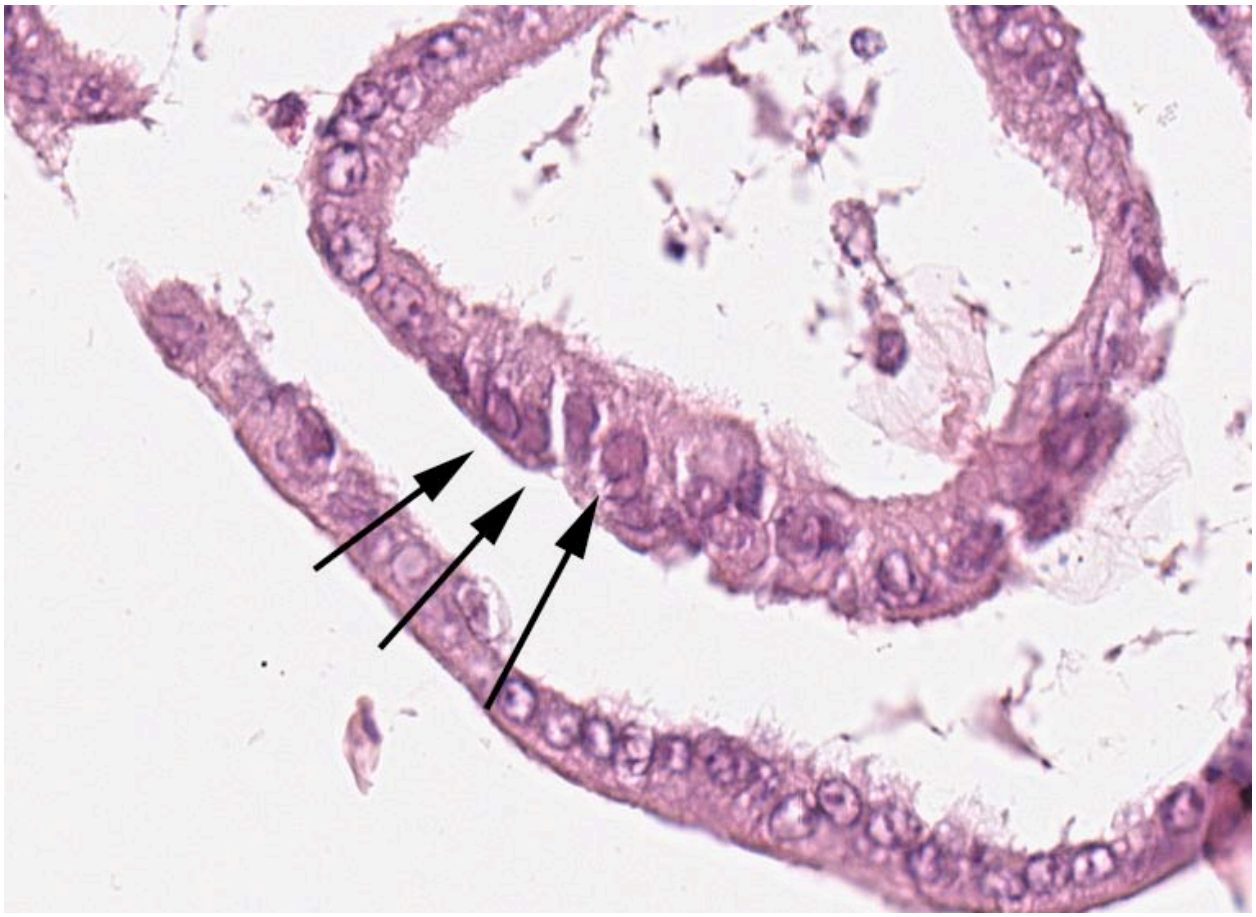
Contributor's Microscopic Description: Lung: Severe and diffuse inflammatory changes involving



1-1. Lung, tortoise. There is general consolidation of faveoli throughout the lung; only the large bronchiole (arrowhead) is partially spared. Pleura (arrows) are markedly expanded by edema. (HE 63X)



1-2. Lung, tortoise. Faveoli are markedly expanded and filled with abundant polymerized fibrin, heterophils, macrophages, and cellular debris. Congested capillaries and smooth muscle show the extent of the faveolar tissue in the photomicrograph. (HE 260X)



1-3. Lung, tortoise. Groups of contiguous faveolar pneumocytes contain nuclei which are expanded by smudgy amphophilic viral inclusions. (HE 400X)

80% of the pulmonary parenchyma are present in all sections. Upper and lower airways (ediculae) contain variably abundant luminal accumulation of mucus and fibrin admixed with numerous sloughed necrotic epithelial cells, moderate numbers of heterophils, viable or occasionally degenerated (karyolysis and karryorrhexis), and rare reactive macrophages. The epithelium lining larger airways and ediculae is multifocally eroded and ulcerated. In the epithelial cells (sloughed and viable), nuclei are multifocally characterized by chromatin margination and occasionally contain amphophilic, homogeneous variably sized and shaped inclusions bodies often filling the nucleus (consistent with herpesviral inclusions).

The pulmonary interstitium is moderately to severely and diffusely expanded by hyperaemia and edema, numerous heterophils and lesser numbers of lymphocytes and plasma cells. Pleura is severely, diffusely edematous and contains a small number of heterophils.

Contributor's Morphologic Diagnosis: Severe, diffuse, acute, necrotizing pneumonia with amphophilic intranuclear inclusions consistent with herpesvirus, Greek tortoise, *Testudo graeca*.

Etiology: Chelonid herpesvirus (most likely type 3).

Contributor's Comment: Herpesviruses are enveloped viruses with a double stranded DNA surrounded by icosahedrally arranged caspomeres. Herpesvirus infections are widespread and occur in most classes of vertebrates including fish, amphibians, and reptiles. Infection with herpesvirus has been reported in chelonians, lizards, snakes and in crocodilians. In chelonians, herpesviruses have been associated with several disease complexes, which are characterized by diphtheritic-necrotizing stomatitis, hepatitis, rhinitis, tracheitis, and pneumonia in tortoises.[8] Tortoises in the genus *Testudo* including Greek tortoise (*T. graeca*), Hermann's tortoise (*T. hermanni*), and Russian tortoise (*Agrionemys horsfieldi*) are particularly prone to infection with Chelonid herpesvirus (ChHV). ChHV infections have been associated with glossitis, stomatitis, enteritis, and meningoencephalitis in Hermann's tortoises (*Testudo hermanni*); with stomatitis and enteritis in Afghan tortoises (*Testudo horsfieldii*); with stomatitis,

tracheitis, and pneumonia in desert tortoises (*Gopherus agasizii*)[11]; and with stomatitis and encephalitis in spur-thighed tortoises (*Testudo graeca*).[8] Green turtles older than one year of age have also been susceptible to pneumonia, tracheitis and conjunctivitis ascribed to herpesvirus.[8]

Herpesviruses have also been associated with oral, respiratory, cutaneous, and genital lesions in Atlantic loggerhead sea turtles (*Caretta caretta*)[13] and skin diseases in sea turtles, such as gray patch disease and fibropapillomatosis in green turtles (*Chelonia mydas*).[1,7,12]

The exact route of transmission of herpesvirus in wild chelonids is still unknown. In captive animals, a major means of transmission is the exchange of pet tortoises between private collections.[8] It is very likely that direct contact between affected animals and unaffected tortoises represents the primary route of transmission. The finding of viral particles in testicular epithelium of Greek and Hermann's tortoises suggests the possibility of vertical transmission.[8]

Herpesviruses identified in various species of turtles and tortoises have been preliminarily named chelonid herpesviruses (ChHVs), but represent an up-to-now unassigned species in the herpesvirus family. Classification of ChHVs is mainly based on putative differences in the host spectrum and 4 variants have been recognized. ChHV-1 was first described in association with gray patch disease in captive green sea turtles (*Chelonia mydas*) in the West Indies.[12] This disease is characterized by patchy gray areas of hyperkeratotic and necrotic papules that occur over the head, neck, and flippers. ChHV-2 was seen in two Pacific pond turtles (*Clemmys marmorata*) with fatal hepatic necrosis. A similar disease has been seen in painted turtles (*Chrysemys picta*) and in map turtles (*Graptemys pseudogeographica*) in association with herpesvirus-like particles.

These viruses have been preliminarily named ChHV-3.[6] ChHV-4 was seen in tissues of Argentinian tortoises (*Geochelone chilensis*) with necrotizing stomatitis or mouth rot. Interestingly, red-footed tortoises (*Geochelone carbonaria*) kept together with the diseased Argentinian tortoises remained clinically healthy.[4] Epizootics of chronic seromucous rhinitis (running nose syndrome) were described in large populations of captive *T. graeca.* This outbreak was part of a series of epidemic ChHV infections that have occurred in Europe during the last decade. In most cases, outbreaks follow shared housing of different tortoise species after addition of new animals. In all of these cases, presumed carrier species, especially *T. graeca,* remained healthy, whereas other, presumably less resistant species, became sick or died.

Clinical signs in tortoises include nasal serous to mucopurulent discharge, open mouth breathing, wheezing, dyspnea, lethargy, anorexia, weight loss and ataxia. Radiographs, magnetic resonance, CT scans, bronchoscopy, and cytology have been used for clinical diagnosis.[8]

Pathological findings in most chelonids with respiratory disease include necrotizing caseous stomatitis that extends in the oral cavity and nares and necrotizing glossitis with presence of diphtheritic plaques. Lower airways can be involved resulting in necrotizing pneumonia and emphysema. Enteritis and hepatomegaly have been also reported.[8] In tortoises, eosinophilic intranuclear inclusions are commonly seen in epithelial cells of affected tissues stained with haematoxylin and eosin and are associated with syncytial cells.[8] Secondary bacterial complications are associated with development of multiple bacterial granulomas. Intranuclear inclusions have also been reported in lung and trachea of green turtles (*Chelonia mydas*) with respiratory disease[5] and in cutaneous fibropapillomas.[7] Using TEM, virions can be detected in the nucleus and cytoplasm of infected cells of the tongue, trachea, bronchi and alveoli, endothelial cells of glomerular capillaries and within neurons and glial cells of the medulla oblongata and diencephalon.[8]

Tests that have been developed to diagnose chelonid herpesvirus include enzyme-linked immunosorbent assay (ELISA) for the detection of herpesvirus antibodies in plasma samples of Mediterranean tortoises.[9] Indirect and direct immunoperoxidase assay have been used either for assessing the presence of anti-herpesvirus antibody in tortoise plasma or for detecting herpesvirus antigen in tissues.[10]

ChHV DNA has been demonstrated in a broad range of formalin fixed and paraffin embedded tissues in tortoises suffering from stomatitis–rhinitis complex by in situ hybridization and PCR. The ISH signal colocalizes to the same areas and cell types that contain intranuclear inclusions in haematoxylin and eosin stained tissue sections from tortoises of different geographic provenances. Nuclear hybridization signals have been detected in epithelial cells of the lingual mucosa and glands, in tracheal epithelium, pneumocytes, hepatocytes, the renal tubular epithelium, cerebral glial cells and neurons, intramural intestinal ganglia and in endothelial cells of many organs.[14]

JPC Diagnosis: Lung: Pneumonia, bronchointerstitial, fibrosing, heterophilic and histiocytic, subacute, diffuse, severe, with type II pneumocyte hypertrophy and exfoliation with numerous epithelial intranuclear viral inclusions.

Conference Comment: The differential diagnosis discussed by conference participants included adenovirus, ranavirus, and fibropapillomavirus. Previous reports of chelonian adenoviruses include an atAdenovirus in a leopard tortoise (*Geocehlone pardalis*) and a Siadenovirus in Sulawesi tortoises (*Indotestudo forsteni*).[15] Reported gross lesions include hepatosplenomegaly, fibrinonecrotic membranes in the lumen of the colon, oronasal fistulae, and ulceration of the tongue and oral mucosa. The histologic appearance of adenovirus is very similar to herpesvirus, including epithelial, endothelial and myeloid necrosis with basophilic to amphophilic intranuclear viral inclusions in many tissues.[3,14]

Ranavirus is in the family Iridovirus, with gross lesions including hepatic necrosis, ulcerative tracheitis, pneumonia, and ulcerative pharyngitis and esophagitis. Histopathologic findings include basophilic intracytoplasmic viral inclusions in hepatocytes and epithelial cell, and fibrinoid vasculitis in multiple organs. Reptiles are thought to aquire ranavirus from amphibians.[3]

Fibropapillomatosis is found in all species of sea turtles except for the leatherback, and is caused by a herpesvirus. Fibropapilloma-associated turtle herpesvirus causes a debilitating disease characterized by large numbers fibropapillomas which result in decreased mobility and occasional blindness when located near the eyes. These are often accompanied by anemia and immunosuppression. Histology of the masses is that of a typical fibropapilloma, and intranuclear viral inclusions are rarely seen. Fibropapillomas can also be seen on the viscera, with the kidney and lung being primary target tissues.[16]

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CASE II: 11-03780 (JPC 4003460).

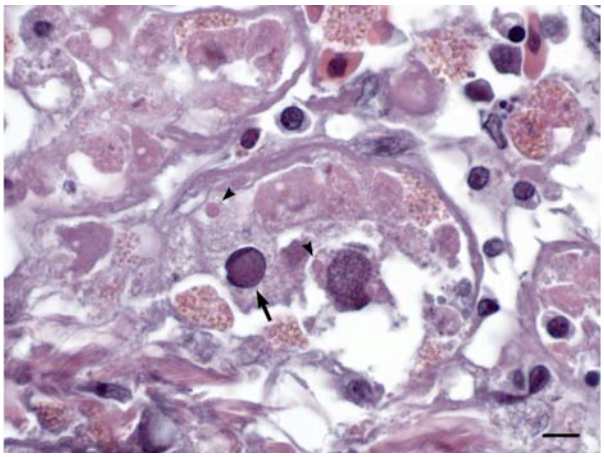
Signalment: An approximately 1-year-old male *Boa constrictor*.

History: Three boid snakes were purchased from a breeder and incorporated into a private reptile collection. Although apparently normal at the time of purchase, within a few days this snake developed sluggish behavior with twisting, ataxia, and opisthotonus. Clinical signs persisted for a week prior to death.

Gross Pathology: The body of an 823 g, 129 cm male boa was in thin body condition with decreased fat stores and mildly atrophied skeletal muscle. A moderate pericardial effusion consisting of 2-3 ml of serous to faintly serosanguinous fluid was present as well as serous atrophy of epicardial fat. Excessive clear mucus was present in the mouth, and the stomach was empty. The kidneys appeared somewhat pale and ureters were prominent and filled with uric acid.

Laboratory Results: Routine fecal flotation revealed no parasite eggs or oocysts.

Contributor's Microscopic Description: Liver: There is widespread hepatocellular necrosis associated with large, amorphic, amphophilic to basophilic intranuclear inclusion bodies resulting in karyomegaly and margination of nuclear chromatin. Occasional hepatocytes and numerous biliary epithelial cells contain one or more rounded, brightly eosinophilic intracytoplasmic inclusion bodies. Loose fibrin thrombi, high numbers of heterophils and scattered lymphocytes are present within sinusoids throughout the liver.



2-1. Liver, snake. A hepatocyte nucleus is markedly expanded by a darkly basophilic cytoplasmic adenoviral inclusion (small arrow). A necrotic hepatocyte is present (large arrow). The remaining hepatocytes are atrophic and numerous heterophils are present within sinusoids. Remaining viable hepatocytes contain brightly eosinophilic round protein inclusions characteristic of boid inclusion disease. (HE 1000X) Photograph courtesy of the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon State University, Magruder Hall 134, Corvallis, OR 97331 http://oregonstate.edu/vetmed/diagnostic

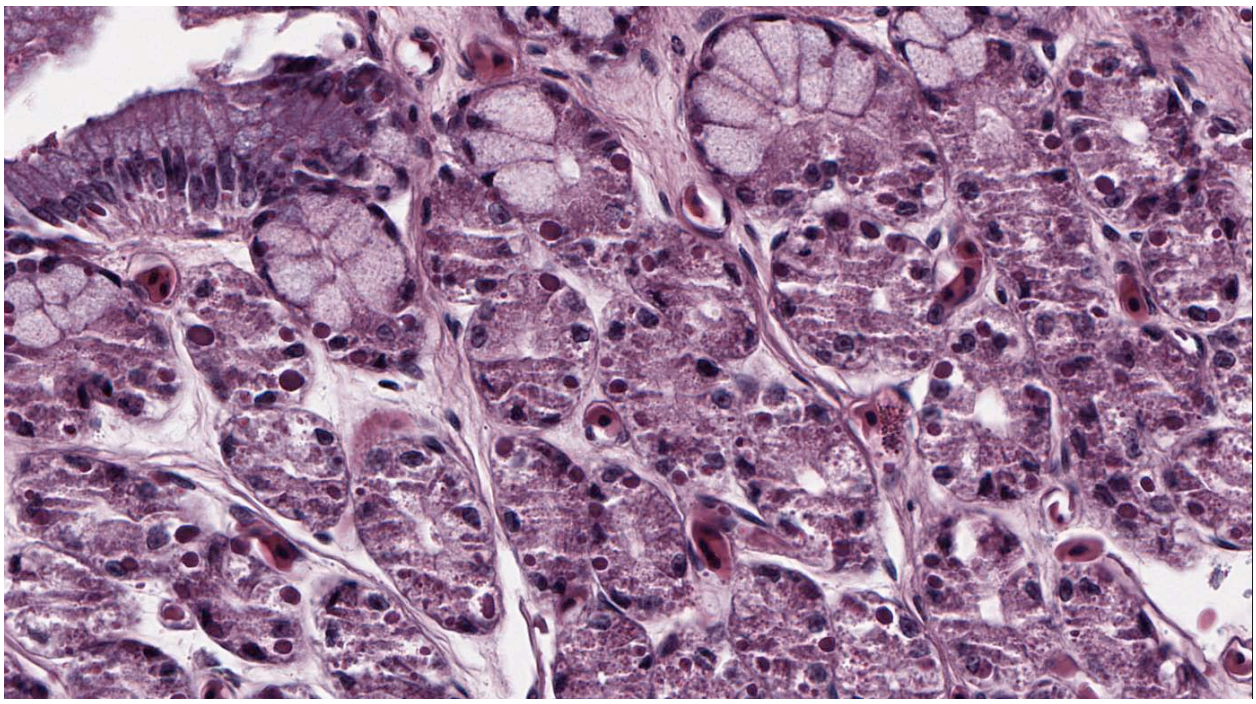
Stomach: Numerous mucosal epithelial cells contain rounded, brightly eosinophilic intracytoplasmic inclusion bodies. Occasional epithelial cells contain amphophilic intranuclear inclusion bodies. Additional lesions not included on the slide include brightly eosinophilic intracytoplasmic inclusion bodies within neurons and glial cells in the brain (occasionally associated with gliosis); within epithelial cells of the adrenal gland, thyroid follicles, bronchi, renal tubules, acinar pancreatic cells, and intestinal enterocytes; within lymphocytes in the spleen; and occasionally within cardiomyocytes. Low numbers of intestinal enterocytes also contain amphophilic intranuclear inclusion bodies.

Contributor's Morphologic Diagnosis: Liver: Severe, diffuse acute necrotizing hepatitis with intralesional inclusion bodies.
Stomach: Intracytoplasmic and intranuclear inclusion bodies.

Contributor's Comment: Death in this case was determined to be due to dual viral infection with histologic lesions consistent with concurrent inclusion body disease and adenovirus infection, although confirmation of the latter infection by virus isolation was not attempted. In the absence of ancillary testing, herpesviral infection could not be ruled out as a cause of hepatic necrosis.

Inclusion body disease is an important infection in boid snakes, having emerged during the last 30 years on several continents.[13] Although snakes may remain chronic carriers of the disease, once clinical signs develop, the disease is fatal within weeks or months.[2,13] Clinical signs are variable and can include neurologic, digestive or respiratory systems.[6,12,13] Antemortem diagnosis can be achieved by biopsy of the liver, esophageal tonsil, gastric mucosa or skin; or by detection of typical cytoplasmic inclusion bodies within peripheral blood leukocytes, although this method yields higher false positives and false negatives.[2,3] Histologically, there are large, brightly eosinophilic intracytoplasmic inclusion bodies within a wide range of tissues (particularly in the liver and pancreas), often in the absence of a tissue or cellular response.[1,2,6,14]

Retroviruses have been isolated from boids with inclusion body disease; however, Koch's postulates have yet to be completely fulfilled.[5,13,16] The bloodsucking snake mite, *Ophionyssus natricis*, may act as a vector.[13] Concurrent infectious disease is common, possibly due to lymphoid depletion and resultant immunosuppression.[6] Pneumonia, ulcerative



2-2. Stomach, snake. Numerous chief cells contain brightly eosinophilic round protein inclusions characteristic of boid inclusion disease. Chief cells are shrunken and lack zymogen granules (atrophy). (HE 400X)

stomatitis[6], entamoebic colitis[10], and neoplastic diseases such as lymphoma[11] have been reported in conjunction with inclusion body disease.

Adenoviral hepatitis has previously been described in boid snakes, and is characterized by severe necrotizing hepatitis with basophilic to eosinophilic intranuclear inclusions within hepatocytes, accompanied by heterophils and small mononuclear cells.[4,8,13] Other lesions previously attributed to adenovirus or adenoviral-like infection in snakes include enteritis, splenitis, nephritis, pneumonia and encephalopathy.[7] The ability for latent adenoviral infections to induce clinical disease following immunosuppression has been demonstrated in species other than snakes[15], and in the case presented here it is tempting to speculate that inclusion body disease was involved in that respect.

Major viral diseases in boid snakes

Agent	Typical lesions	Inclusions
Adenovirus	Necrotizing hepatitis	Amphophilic (occasionally eosinophilic), intranuclear
Herpesvirus	Necrotizing hepatitis, glomerulonephritis	Eosinophilic to amphophilic intranuclear inclusions
Inclusion body disease virus (suspected retrovirus)	Inclusions in the absence of a tissue or cellular response	Eosinophilic, intracytoplasmic
Paramyxovirus	Hemorrhagic to necrotizing pneumonia, pancreatitis, occasionally CNS disease	Occasional, eosinophilic, intranuclear or cytoplasmic
Adenovirus, parvovirus, picornavirus, herpesvirus	Viral-associated gastrointestinal disease - causal relationship not established	

JPC Diagnosis: 1. Liver: Hepatic atrophy, diffuse, severe.
2. Liver: Necrosis, hepatocellular, multifocal, with rare intranuclear viral inclusions.
3. All cell types: Intracytoplasmic inclusions.
4. Stomach: Diffuse loss of parietal cell granules.

Conference Comment: The moderator attributed the hepatic atrophy and gastric parietal cell granular degeneration to the poor nutritional status of the animal, which is a common finding in inclusion body disease. The rare intranuclear inclusions in hepatocytes are likely due to the concurrent adenoviral infection. The conference moderator also noted the presence of melanin pigment within the liver, which is normal in reptiles.

The moderator discussed the composition of the inclusions seen in inclusion body disease, which remains controversial. The inclusion material was initially thought to result from the associated retrovirus, but recent research suggests that this condition may be a storage disease, similar to the transmissible spongiform encephalopathies. The intracytoplasmic inclusions, which ultrastructurally are either non-membrane limited aggregates of granular

electron-dense material, or membrane-bound aggregates of electron-dense material with membrane-like fragments, are composed of a distinct 68-kd protein, and typically cause no inflammatory response, as opposed to necrosis and inflammation associated with viral infections. Nonviral inclusions have been demonstrated in cells infected with other retroviruses, such as avian leukosis virus, visna virus, and simian immunodeficiency virus.

Boid inclusions stain with PTAH and toluidine blue, and are eosinophilic with H&E, suggesting protein composition; they do not stain with Congo red or thioflaven-T, suggesting that the material in not amyloid. Boid inclusions have a similar staining pattern to Mallory bodies, non-viral inclusions composed of cytokeratin filaments. The inclusions may also be overproduced or poorly degraded components of the virus that accumulates in the host cell cytoplasm.[16]

As this is one of the most important viral diseases of boas and pythons with no known treatment, diagnosis of IBD is imperative. Antemortem diagnosis is often easily obtained through biopsy, since the inclusions are present in all tissues. Preferring the liver, the moderator discussed various high-yield biopsy locations. The skin is a poorer option because inclusions are more widely distributed, necessitating multiple biopsies to decrease false positive results. Cytology is another option, but the slides must be stained with H&E in order to differentiate the inclusions from other structures, such as lipid or intracellular parasites. Currently, immunohistochemical staining is available for frozen tissue, which may lead to a serologic test in the future.

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CASE III: 08120419 (JPC 3136279).

Signalment: 3-year-old female red kangaroo (*Macropus rufus*).

History: This kangaroo died acutely with no observed clinical signs. Four animals died at this private zoo over the past 3 months, also without any clinical signs. This zoo has a large feral cat population.

Gross Pathology: The animal was in a mild state of decomposition. Gross examination revealed the animal was in good nutritional condition. The lungs failed to collapse upon opening the thoracic cavity. There were hundreds of firm, 1-2 mm in diameter, white to tan, slightly raised foci disseminated throughout the pulmonary parenchyma. Mediastinal and tracheobronchial lymph nodes were enlarged and on section were hemorrhagic. Multiple white radiating streaks were identified on the epicardial surface extending into the myocardium.

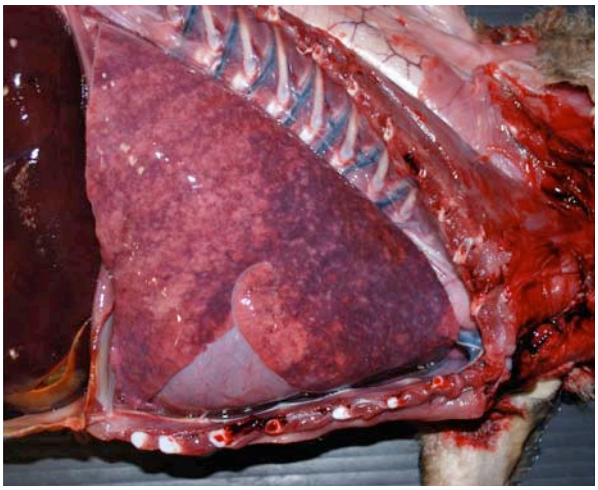
Laboratory Results: Immunohistochemical stains for *Toxoplasma* on lung tissue were positive. Fluorescent antibody staining for CAV-1 on lung tissue was negative.

Contributor's Microscopic Description: Lung: The normal architecture of the lung is disrupted by interstitial and alveolar inflammation, necrosis, fibrin deposition, edema, and intralesional protozoa. Expanding and occasionally forming nodular aggregates within the alveolar interstitium and alveoli are a large number of macrophages, lymphocytes, and plasma cells. Rare degenerate neutrophils are observed. Often the alveolar interstitium contains abundant amounts of fibrin. There is marked necrosis within the sections, characterized by eosinophilic cellular debris and basophilic nuclear debris. Admixed within the inflammation are 1-2µm, oval to round organisms arranged individually (tachyzoites) or grouped together into packets or cysts (bradyzoites) 20-25 µm in diameter. There is a mild amount of pulmonary edema. Alveolar interstitium are markedly congested. [*Organisms are present in all slides; conspicuous in some, inconspicuous in others*]

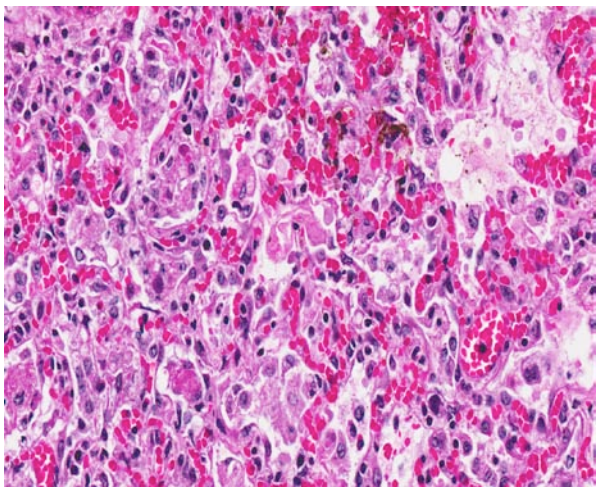
Contributor's Morphologic Diagnosis: Lung: Necrotizing interstitial pneumonia, lymphohistiocytic, diffuse, acute, severe, with multifocal necrosis and intralesional *Toxoplasma gondii* protozoa.

Contributor's Comment: Toxoplasmosis is caused by *Toxoplasma gondii*, an obligate intracellular coccidian parasite. *Toxoplasma gondii* has a broad intermediate host range and the only known definitive hosts for *T. gondii* are domestic cats and other felidae.[2] Infection with *T. gondii* within the cat is dependent on enteroepithelial or extraintestinal life cycle. In kangaroos, similar to other intermediate hosts, infection is dependent on the extraintestinal life cycle. *Toxoplasma gondii* has a worldwide distribution and causes high morbidity and mortality in Australian marsupials, particularly macropods.[1]

Kangaroos are herbivores and presumably acquire *T. gondii* by ingestion of oocysts from their environment. In this case, it was difficult to determine whether there was contamination of the foods, grazing areas, or water, or if oocysts were ingested during grooming activities. However, it was likely that feral cats defecated in the enclosure area, resulting in contamination of the ground or feeding stations



3-1. Lung, kangaroo. Disseminated throughout the non-collapsing lungs were hundreds of firm 1-2 mm in diameter, white to tan, slightly raised foci. Photograph courtesy of Oklahoma State University, Department of Veterinary Pathobiology, 250 McElroy Hall, Stillwater, OK 74078. www.cvm.okstate.edu



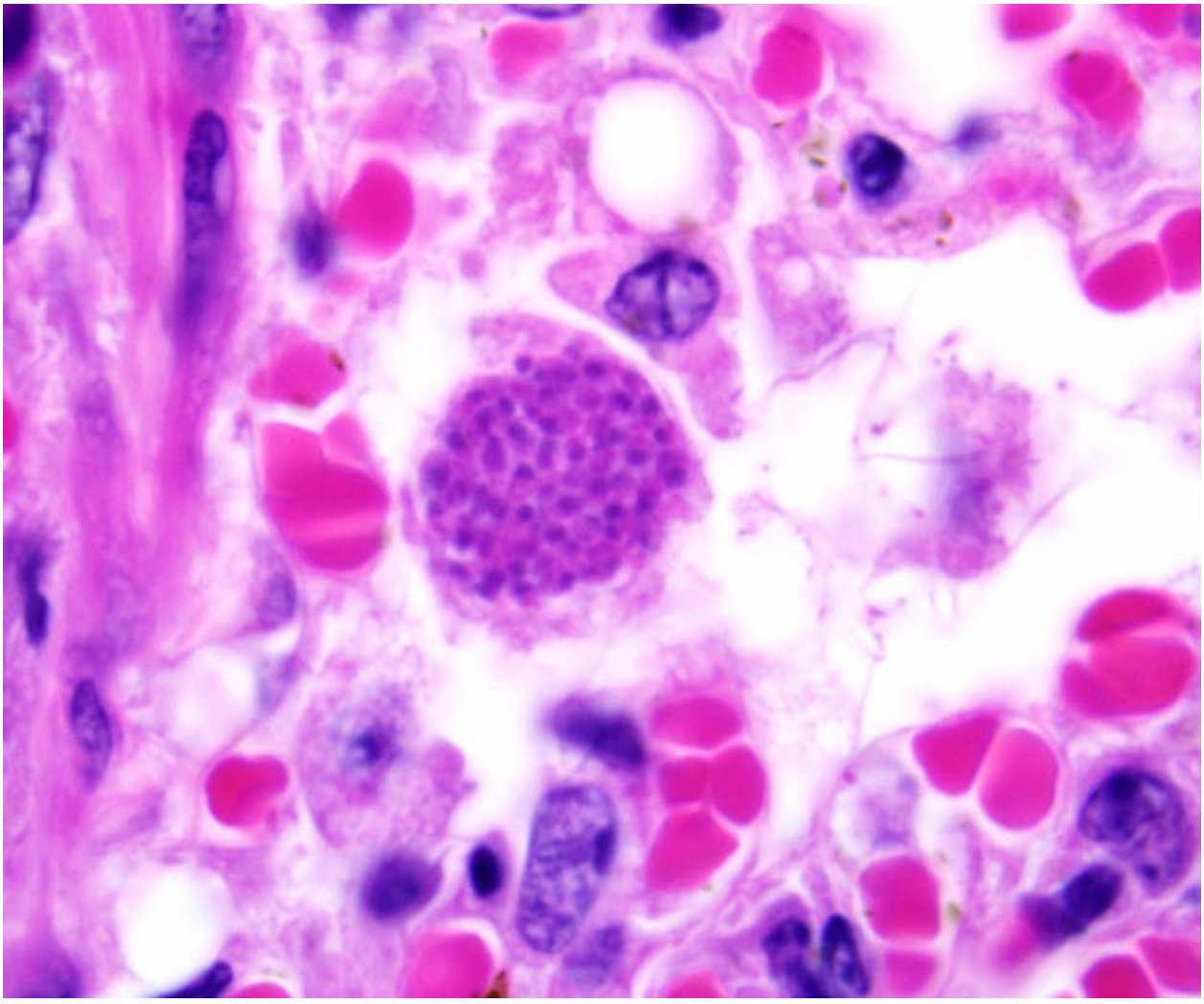
3.2. Lung, kangaroo. Alveolar septa are markedly expanded by fibrin and edema, and occasionally type I pneumocytes are necrotic. Alveoli are filled with polymerized fibrin, foamy alveolar macrophages, neutrophils, and small amounts of cellular debris. (HE 400X)

potentially exposing these animals to infectious oocysts.

At necropsy, kangaroos with toxoplasmosis have gross lesions similar to other domestic animal species; including pulmonary congestion and edema, pulmonary consolidation, pale myocardial streaks and hemorrhage, a miliary pattern of white pulmonary foci, lymphadenomegaly, and splenomegaly. In addition to the lung lesion, this patient exhibited inflammatory lesions with *Toxoplasma* organisms in the brain, heart, liver and kidney. Similarly, disseminated toxoplasmosis was confirmed in the other four kangaroos that had died previously.

JPC Diagnosis: Lung: Pneumonia, interstitial, necrotizing, diffuse, moderate, with rare intrahistiocytic apicomplexan cysts and free zoites.

Conference Comment: A characteristic finding of toxoplasmosis is necrosis, which is caused both by rupture of infected cell membranes by rapidly dividing tachyzoites, and by ischemia secondary to vasculitis. The moderator emphasized that the most susceptible captive zoo species are the squirrel monkey, macropods, lemurs, and the Thomson's gazelle, that protozoal cysts are most readily found in skeletal muscle and the central nervous system, and that pulmonary infection is the most common cause of death. Characteristic gross findings in the lung include pulmonary consolidation with multifocal to coalescing green foci; lymphadenomegaly, and hemorrhage and necrosis of the lymph nodes are also commonly noted. The moderator also discussed the serious problem that toxoplasmosis causes in zoos, with the parasite reservoirs being feral cats, opossums, rats, and cockroaches.



3-3. Lung, kangaroo. Alveolar macrophage containing numerous rutracytoplasmic zoites consistant with Toxoplasma gondi. (HE, 1000 X)

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CASE IV: N-0811520 (JPC 3134355).

Signalment: 1-year-old male giant elephant shrew (*Rhynchocyon petersi*).

History: A 1-year-old male captive-bred giant elephant shrew (*Rhynchocyon petersi*) presented with lameness of the right hind limb. The shrew had been pair housed with a male littermate in a 75 sq. ft. indoor enclosure since arriving 3 months earlier from the Smithsonian National Zoological Park. Empirical daily therapy with oral meloxicam (0.1 mg/kg) and a ten-day course of cefdinir (10 mg/kg) resulted in minimal improvement. Physical exam and radiographs revealed no remarkable findings. Three weeks later, the fifth digit of the left forelimb exhibited marked swelling. Further, a mass measuring 2 x 2 x 0.5 cm was palpated at the medial and lateral aspect of the right stifle. Subsequent, repeat radiographs of the affected digit (5th phalanges) and right stifle (distal femur and proximal tibia) revealed bone lysis and soft tissue swelling. Two weeks after limited success with oral antimycobacterial therapy using rifabutin (20 mg/kg) and azithromycin (15 mg/kg), the shrew became ataxic, lethargic and anorexic. Shortly after transport to an intensive care unit for supportive care, the shrew was found tachypneic in right lateral recumbency, and due to its deteriorating condition and poor prognosis, the elephant shrew was humanely euthanized and submitted for necropsy.

Gross Pathology: On gross examination, there was enlargement of the right femorotibial (stifle) joint with multiple, coalescing foci of purulent material (abscesses) within a 2 cm x 2 cm x 1.5 cm area of the quadriceps and a 3 cm x 2 cm area of the hamstring muscles. Crepitus suggestive of a fracture was present on palpation of the stifle joint. The fifth digit on the left front lateral toe was swollen. Numerous pinpoint to 0.2 cm white foci were found disseminated throughout all lung lobes and diffuse, pinpoint, white foci were frequently observed on liver surfaces. Approximately 2 ml of serous sanguineous fluid was observed in the abdominal cavity.

Laboratory Results:
Microbiology Culture:
Right stifle joint and lung: *Mycobacterium spp*.
PCR:
A 100% identity for *Mycobacterium intracellulare*

Contributor's Microscopic Description: Lung: Within ~25% of the lung lobes, there are multiple individual to coalescing irregular granulomas composed centrally of necrosis with a peripheral rim of degenerative neutrophils, which in turn is surrounded by numerous macrophages, scattered multinucleated giant cells and occasional lymphocytes and plasma cells. Some granulomas encompass airways and vasculature with extension of inflammatory cells into the surrounding tissue. The pulmonary interstitium is also multifocally mildly to severely expanded by macrophages and neutrophils spatially distinct from granulomas. There are also multifocal areas within the airways that contain macrophages with multifocal loss of airway epithelium. Review of acid fast bacteria (AFB)-stained sections of lung and stifle joint revealed numerous AF-positive bacteria.

Contributor's Morphologic Diagnosis: Lung: Pneumonia, pyogranulomatous, severe, chronic active, multifocal.

Contributor's Comment: The giant elephant-shrew (*Rhynchocyon petersi*) is a small insectivorous mammal of the family *Macroscelididae*, widely distributed across central and southern Africa. Their traditional common English name comes from a resemblance between their long noses and trunk of an elephant, and an assumed relationship with the true shrews (family *Soricidae*) in the order Insectivora. Phylogentically, elephant shrews are closely related to aardvarks, golden moles and tenrecs. Currently, there are 15 known species in this strictly African mammal group, three of which are referred to as "giant" elephant shrews belonging to the genus *Rhynchocyon*. All three *Rhynchocyon* species are considered threatened due to habitat destruction and fragmentation.

Mycobacterium avium complexes (MAC) are a group of nontuberculous mycobacteria characterized as gram-positive, aerobic, non-motile, acid-fast rods. *M. avium* and *M. intracellulare* are the most notable species within the group.[30] Because *M. avium ssp. avium* and *M. intracellulare* are difficult to distinguish on the basis of phenotypic characteristics, the term *M. avium* complex is used when referred to these organisms. The mycobacterium now designated as *M. intracellulare* was first cultured in 1969 from the sputum of tuberculosis patients in the Battey State Hospital of Rome, Georgia and was originally referred to as "Battey bacillus".[25] In 1971, the nontuberculosis mycobacteria were divided into three groups; based on their association with human disease, production of yellow or orange pigment, and their rate of growth. The photochromogens contain 3 species that develop yellow or orange pigment when exposed to light. Examples include *M. kansasii*, *M. marinu, and M. simiae*. The scotochromogens, including *M. gordonae, M. szulgai* and *M. scrofulaceum,* form orange-yellow pigment in the dark. The unpigmented *M. avium* complex (MAC)*, M. xenopi,* and *M. malmoense* have been classified as nonphotochromogens.[25] Based on glycolipid typing MAC has been further subdivided

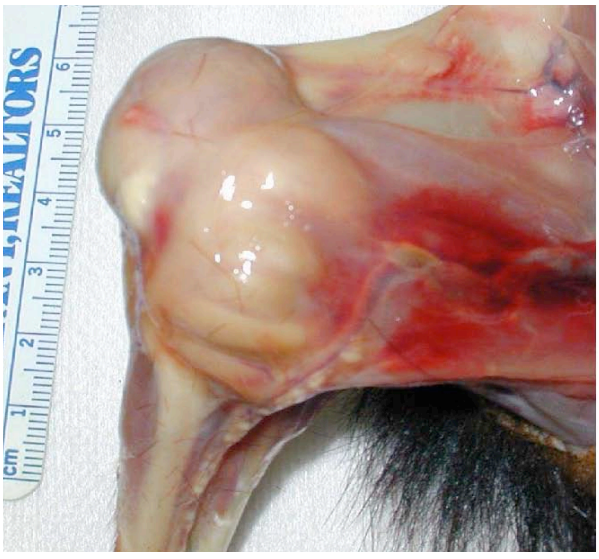
into 28 serotypes.[28] Serotypes 1 to 6 and 8 belong to *M. avium* and serotypes 7, 12, 14, 16, and 18 to *M. intracellulare.*

Rodents, insectivores, domestic animals and humans can be a reservoir of mycobacteria. The occurrence of mycobacteria in rodents was first reported by Wells and Oxon[5], with a prevalence of 9 to 31% of *Mycobacterium microti* in rodents. Mycobacteriosis in shrews was also demonstrated by the isolation of the organism from tuberculous pathomorphological changes in the lungs, spleen, liver, kidneys and lymph nodes in 8 of 500 common shrews (*Sorex araneus*) caught in 1946 in Great Britain.[5] Mycobacteria are found in the feed and bedding, as well as the droppings of livestock.[9]. Insectivores and small rodents commonly come into contact with mycobacteria via plants, animal foods, watery environments or contact with birds or humans infected with MAC. At the same time, shrews can easily get inside human or livestock housing and contaminate the environment with their droppings. *Mycobacterium avium-intracellulare* is a common cause of disseminated disease among patients with human immunodeficiency virus (HIV) infection.[3] In a recent study, Voles (*Microtus pennsylvanicus*) infected with *M. bovis* were capable of disseminating the organism via their feces. Both *M. intracellulare* and *M. avium* are capable of gastric transit in animals without destruction due to their resistance to acid. In this way they can be spread over long distances with the migration of the animal.[6] Also, there are several other possible routes of mycobacterial transmission: a) through direct contact with rodent excreta, b) ingestion of food or water contaminated with rodent excreta, c) ingestion of the animal itself, d) inhaling aerosolized rodent excreta, or e) rodent bites, or through ectoparasites.[9]

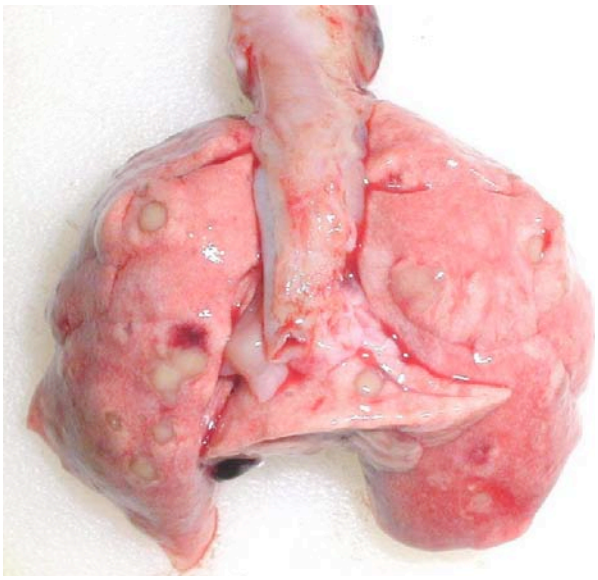
Depending on the route of infection, affected mammals can present with local cutaneous disease or systemic signs related to the alimentary, and/or respiratory tracts. Typically, disseminated MAC disease begins as a localized process that progress rapidly to include numerous organ systems (i.e. pulmonary, central nervous system, lymphatic, gastrointestinal, and musculoskeletal system).[2-4,7,9-12,14,16-19,21-22,24] The common clinical signs of a MAC infection in immune compromised humans are respiratory distress[19,26], anemia, chronic diarrhea, weight-loss[14], lymphadenitis[19,23], lethargy, hepatosplenomegaly, and arthritis.[29,31]

MAC infections must be differentiated from *M. tuberculosis* or *M. bovis*, and other nontuberculous mycobacterial infections, as well as histoplasmosis, cryptococcosis, coccidioidomycosis, and blastomycosis. The rapid speciation and prevalence estimation of these infecting organisms is desirable because of the public health concerns associated with *M. avium intracellulare*, *M. bovis*, and *M. tuberculosis* infections.[27] Thus, laboratory diagnoses by culture, PCR, and /or histopathology are the traditional methods of testing. A positive culture of MAC from normally sterile sites (blood, bone marrow, lymph node, or liver biopsies), should be considered diagnostic of disseminated disease.[21]

4-1. Giant elephant shrew at necropsy – note swelling on the fifth digit on the left front foot. Photograph courtesy of the Section of Comparative Medicine, Yale Medical School 123 LSOG, http://medicine.yale.edu/compmed/



4-2. Quadriceps muscles, giant elephant shrew at necropsy. There are multiple, coalescing foci of purulent material (abscesses) within the quadriceps and hamstring muscles. Photograph courtesy of the Section of Comparative Medicine, Yale Medical School 123 LSOG, http://medicine.yale.edu/compmed/



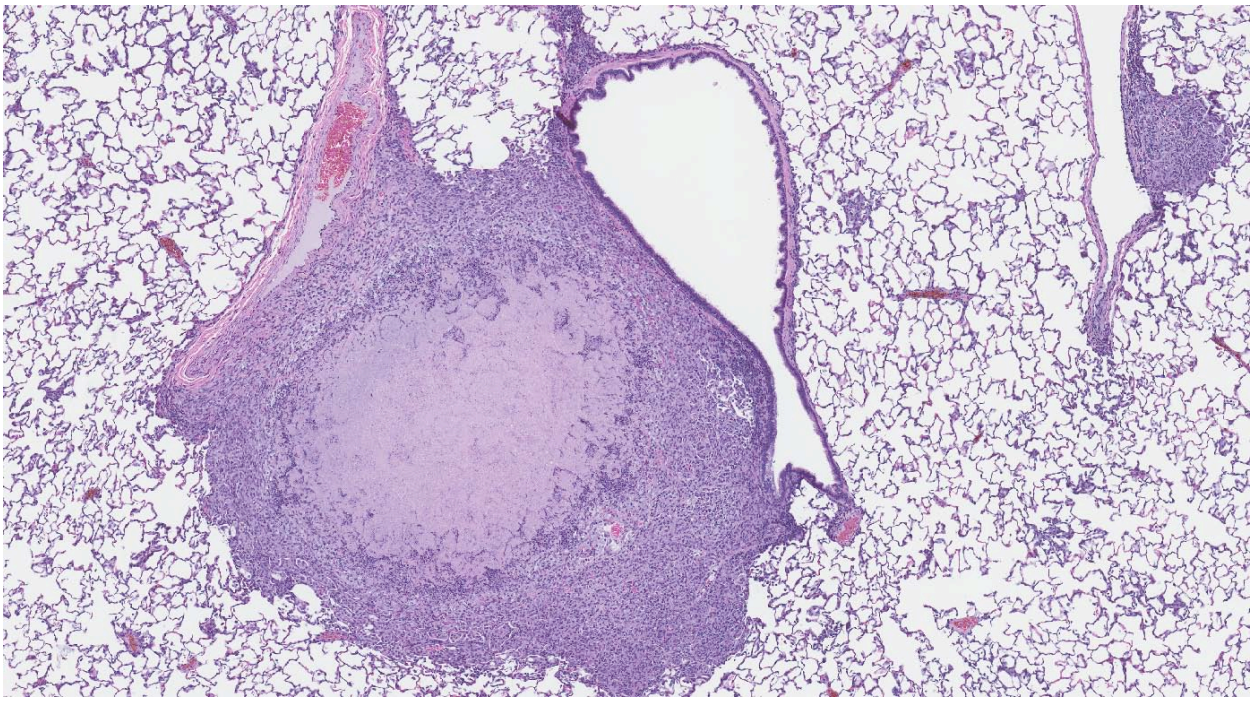
4-3. Lungs, giant elephant shrew. Multiple granulomatous foci are scattered throughout the lung. Photograph courtesy of the Section of Comparative Medicine, Yale Medical School 123 LSOG, http://medicine.yale.edu/compmed/

In this case, one of three possible mechanisms probably explains the occurrence of disseminated MAC. The first possibility is that MAC was introduced directly into the tissue via direct inoculation, then disseminating to various organs over a period of time. Secondly, MAC is ubiquitous in the environment, including water sources, and can contaminate drinking water.[18] Water distribution systems have been reported as a possible source of infection in hospitals, homes, and commercial buildings.[1] Because MAC grows slowly, a six month lag between inoculation and clinical presentation, as seen in humans, is biologically plausible.[13] The third possibility is that the shrew had undiagnosed disseminated MAC at the time of arrival, and underwent a period of stress, which suppressed the immune system. Stress of any kind can tip the scale in the direction of the mycobacteria and produce fulminant infection. Systemic mycobacterial infection is an important differential diagnosis for an acute onset of lameness in insectivores.

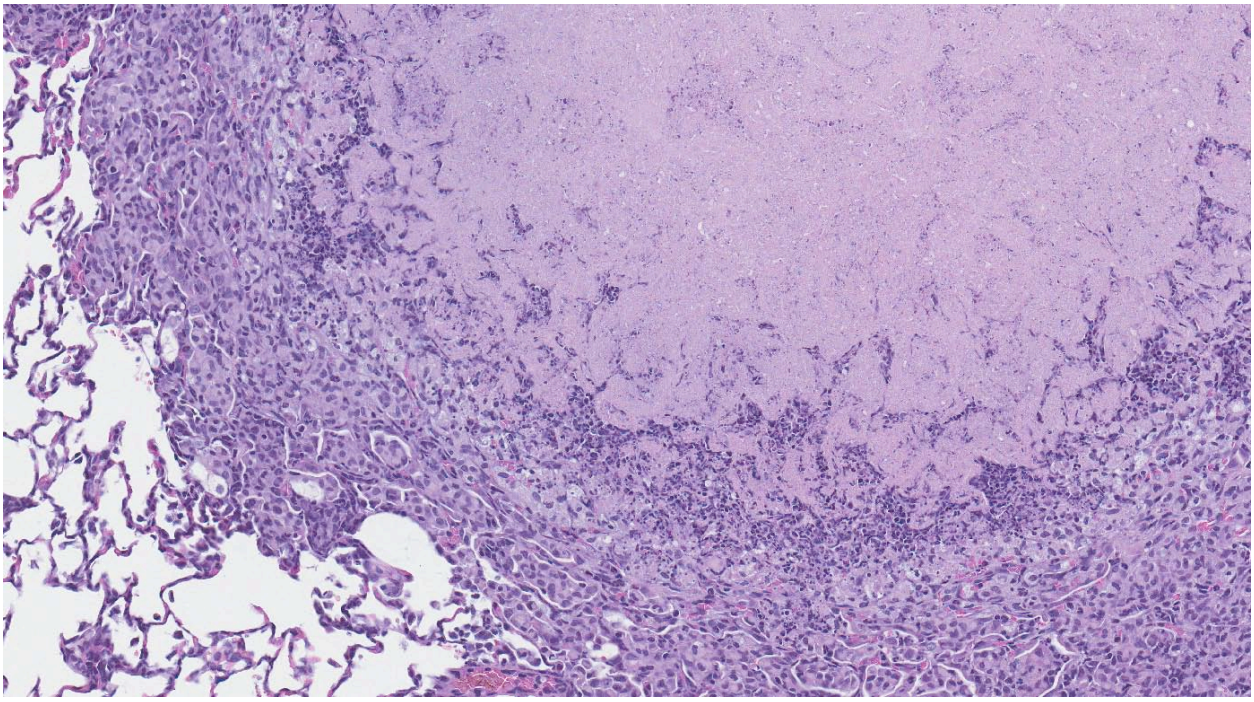
JPC Diagnosis: Lung: Pneumonia, granulomatous and caseating, multifocal to coalescing, moderate, with numerous intracellular bacilli.

Conference Comment: This case does not have discrete granulomas, but manifests rather as nodular aggregates of granulomatous inflammation that appear to spread from alveolus to alveolus. Caseous necrosis is not typical in granulomatous disease without the formation of discrete granulomas; however, conference participants felt that the morphology of the lesions in this case of MAC-induced nontuberculous mycobacteriosis supported granulomatous as a more descriptive and appropriate term.

In contrast to nontuberculous mycobacterial infections such as MAC, *Mycobacterium tuberculosis* is the prototypical etiologic agent resulting in granulomas



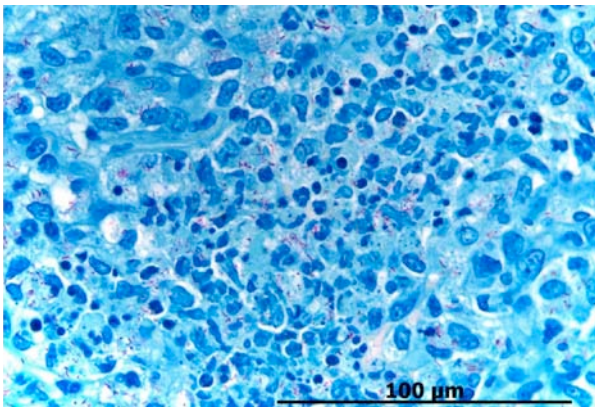
4-4. Lung, giant elephant shrew. Multiple foci of granulomatous inflammation with necrotic centers are present throughout the section. (HE 120X)



4-5. Lung, giant elephant shrew. The advancing edge of the inflammatory nodule is composed of aggregates of epithelioid macrophages which fill alveoli. The characteristic structure of a granuloma, a fibrous wall, is not present in this case, hence the morphologic diagnosis of granuloma is not appropriate in this case. (HE 280X)

and subsequent tubercle formation. Tubercles form when granulomas develop and coalesce. The center of a tubercle typically consist of white, friable material (caseous necrotic debris) derived from lysed cells and bound by epithelioid macrophages, multinucleated giant macrophages, lymphocytes, plasma cells, fibroblasts and collagen. With time, tubercles may become calcified.

Generally speaking, granulomas form as the result of cell-mediated immunity to a poorly degradable entity such as *Mycobacteria*. Initially, antigen presenting cells (APC), such as alveolar macrophages, present phagocytized mycobacterial antigen and MHC class II proteins to naïve T-cells. Binding of the T-cell receptor with mycobacterial antigen and exposure to the cytokine IL-12 released by the APC promotes differentiation from naïve CD4+ TH1 lymphocytes to activated T-helper 1 lymphocytes producing IL-2, which, in turn, activates additional T cells, tumor necrosis factor, and interferon-γ. Interferon-γ is an important factor in the differentiation of monocytes to epithelioid macrophages and multinucleated giant cells.[17]



4-6. Lung, giant elephant shrew. Epithelioid macrophages contain few to moderate numbers of acid-fast bacilli. (Ziehl-Nielsen, 400X)

Contributor: Yale Medical School 123 LSOG
Section of Comparative Medicine
http://medicine.yale.edu/compmed/

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