The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 17

13 February 2008

Moderator:

Dr. Tabitha Viner, DVM, DACVP

CASE I – ND1 (AFIP 3065879).

Signalment: 4-year-old, female, River Otter (*Lutra canadensis*)

History: Found dead on display at the local zoo. The body was found under water.

Gross Pathology: The referring veterinarian performed the necropsy and reported pulmonary congestion, multifocal areas of pallor on the surface of the liver, and thickened myocardium.

Histopathologic Description: Liver sections show unencapsulated, moderately well-defined inflammatory foci characterized by infiltrates of eosinophils, lymphocytes, macrophages and multinucleate giant cells which affect approximately 10% of the tissue **(Fig. 1-1).** Within these inflammatory foci are occasional curvilinear nematode parasites with discernable nuclear columns consistent with microfilarial forms **(Fig. 1-2).** Smaller, primarily eosinophilic foci are randomly present within the parenchyma as well. Periportal zones contain increased numbers of mononuclear cells.

Contributor's Morphologic Diagnosis: Hepatitis, eosinophilic and granulomatous, multifocal, marked with intralesional microfilaria.

Contributor's Comment: *Dirofilaria immitis* infection has been previously reported in the River otter (*Lutra canadensis*), however it is not known if the species serves as a definitive host for the parasite.[8] This is in contrast to the Eurasian otter (*Lutra lutra*) which appears to be able to support the filarial infection.[3] The gross necropsy in this case was performed by the referring veterinarian who did not observe adult worms. However, numerous microfilaria were observed histologically within pulmonary and myocardial blood vessels. Eosinophilic granulomatous hepatitis has been reported in River otters previously, therefore the lesion may be a manifestation of the infection in this species.[6] The parasite was not speciated, however is consistent with the genus *Dirofilaria*.

Specific syndromes associated with heartworm disease include asymptomatic infection, glomerulonephritis, allergic pneumonitis, eosinophilic granulomatosis, pulmonary embolization, congestive heart failure, caval syndrome, and aberrant migration.[1] Changes in this case to correlate best with eosinophilic granulomatosis, however the organ most severely affected was the liver, not the lung. Microfilaria lodged in the sinusoids causing a striking inflammatory response. While microfilaria were clearly present in the circulation, similar foci of inflammation were not observed in lung, kidney or heart, suggesting that the liver may be predilection site for this infection in otters.

AFIP Diagn osis: 1. Liver: Hepatitis, granulomatous, eosinophilic, multifocal to coalescing, moderate, with hepatocellular degeneration and microfilaria, River otter (*Lutra canadensis*), carnivore.
2. Liver, hepatocytes: Vacuolar change, glycogen-type, diffuse, mild.

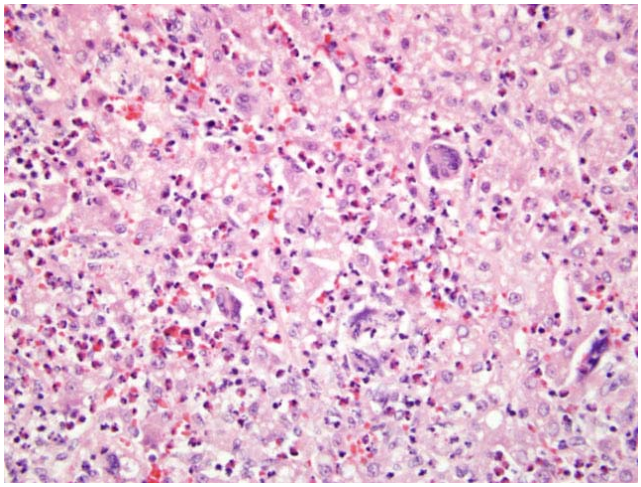
Conference Comment : It is not possible to determine the genus and species of an organism through examination of microfilaria only in tissue cross sections. Body length, the shape of the head and tail, presence or absence of a sheath, and curvature of the body are all used to aid in identification, and even then that may not be sufficient to classify even to the genus level.[9] Obtaining an entire microfilarial organism from a blood or tissue specimen, examination through antigen or antibody tests on serum samples from infected animals or PCR on microfilaria may all aid in identification. Even with the presence of adult worms, their specific speciation may be difficult to impossible if the parasite has not been well described and characterized.[7]

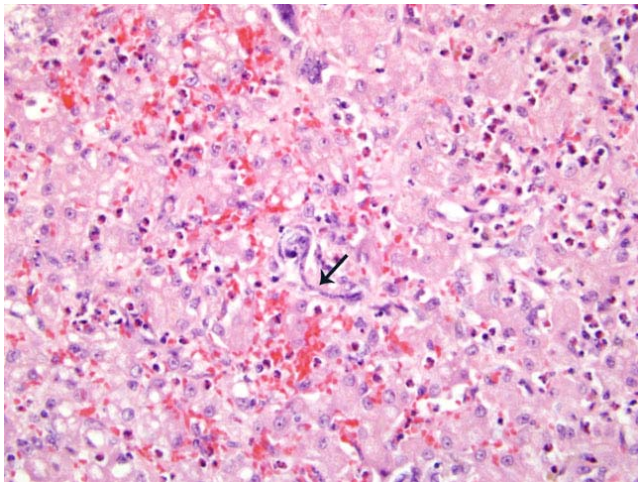
There are numerous species of Dirofilaria that primarily infect particular host species. The most well known cause of microfilariasis of animals in North America is *Dirofuilaria i mmitis*, which is primarily found in the dog.[4] With the exception of *D. i mmitis*, most species of Dirofilaria adults are found within the subcutaneous tissues.[7] Morbidity and mortality associated with *D. i mmitis* infections have been reported in mustelids, canids, felids, otariids, and other domestic and nondomestic carnivores[6], including the river otter.[8] *D. lu trae* infections have only been reported in North American river otters, with adults occurring in subcutaneous spaces and very rarely in the cardiopulmonary vasculature.[6,7] Adult forms of *D. repens* are found within the subcutaneous tissues of canines, other carnivores and occasionally humans.[4] *D. tenuis* is primarily found in raccoons of the southern USA, and *D. striata* within bobcats.

Characteristic histopathologic lesions in the lungs of dogs with dirofilariasis include villous endarteritis with luminal occlusion caused by villous intimal proliferation and medial hypertrophy.[2] Nitric oxide (NO) production has been implicated in the inflammatory response during filarial infections.[5] NO production can be induced by a recombinant *Wolbachia* surface protein.[5] *Wolbachia* is an intracellular endosymbiont bacteria that resides within some filarial organisms, including *Dirofilaria immitis*.[4]

In a recent Journal of Wildlife Diseases article, an unidentified filarial organism was isolated from wild populations of the black-footed ferret.[9] Although this unidentified microfilaria elicited a positive reaction to ELISAs for *D. immitis*, it only shared approximately 76% molecular identity with *D. immitis*, while sharing approximately 97% identity with *Acanthocheilonema viteae*.[9]

Contributor: North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND, 58105
http://www.vdl.ndsu.edu/





1-1. Liver, river otter. Inflammatory infiltrate characterized by eosinophils, lymphocytes, macrophages and multinucleate giant cells. (H&E 400X).
1-2. Liver, river otter. Microfilarid nematode within focus of inflammation (arrow). (H&E 400X).

Table 1-1. Morphologic characteristics of some microfilariae of onchocercids adapted from Wisely et. al.[9]

Filarial species	Length µm	Sheath	Host
Dirofilaria immitis	233-322	no	Dog
Dirofilaria striata	230-371	no	Bobcat
Dipetalonema reconditum	270-290	no	Dog
Loaina uniformis	285	yes	Eastern cottontail
Dirofilariaeformia pulmoni	213-288	no	Eastern gray squirrel
Brugia beaveri	300	yes	Raccoon
Brugia lepori	210-330	yes	Swamp rabbit
Mansonella llewellyni	290 ± 5	no	Raccoon
Monsonella interstitium	250 ± 5	no	Eastern gray squirrel
Molinema arbuta	280-297	no	North American porcupine
Acanthocheilonema reconditum	269-283	no	Dog
Acanthocheilonema mephitis	186-218	no	Striped skunk

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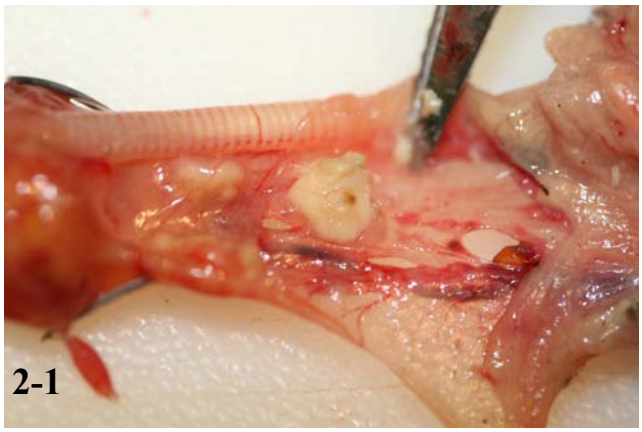
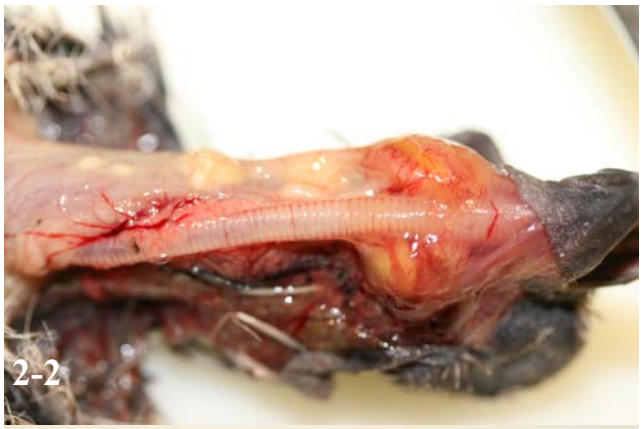
CASE II – X8593 (AFIP 3075563).

Signalment: Juvenile mourning dove (*Zenaida macroura*), Columbiforme

History: Found dead on Zoo grounds

Gross Pa thology: A white, caseous, 5mm diameter mass is apparent within the oral cavity extending into the pharynx. Ventral to the mandible there are two bilateral,





5mm diameter caseous nodules on either side of the trachea. Within the esophagus on the mucosal surface there are multifocal, 3mm diameter plaques of white caseous material **(Fig. 2-1, 2-2, 2-3).**

Histopathologic D escription: Esophagus: In a focally extensive area, the esophageal epithelium is ulcerated and there is a transmural infiltration of inflammatory cells. Replacing the epithelium is a band of macrophages interspersed with 5 – 7 um diameter, ovoid to round protozoa, and a mat of bacteria at the luminal surface. Subjacent to this layer are many heterophils with few macrophages, necrotic debris and scattered protozoa. This inflammatory combination extends through the tunica muscularis to the serosa, which is expanded by heterophils and moderate amounts of mucinous material. The adjacent epithelium is moderately acanthotic with prominent intracellular edema. The serosa below the adjacent epithelium is expanded by a proliferation of capillaries with plump endothelial cells and diffusely the serosa is expanded by heterophils and mucinous material. Small clusters of macrophages, lymphocytes, and few plasma cells are present in the mucosal connective tissue of the remaining, intact esophageal mucosa.
Trachea: Essentially normal tissue for a juvenile bird with unmineralized tracheal rings.

Contributor's Morpho logic Diagn osis: Esophagus: Esophagitis, histiocytic, heterophilic and necrotizing, acute, focally extensive and transmural, severe, with intralesional protozoa, mourning dove (*Zenaitha macroura*), columbiform, avian

Contributor's Comme nt: *Trichomonas gallinae* is carried subclinically by rock doves (*Columba livia*)[5] but may become pathogenic in immunocompromised individuals and mourning doves. The characteristic "canker", or caseous plaque of the dove oral cavity, esophagus, and crop, may also be seen in birds that prey upon or scavenge dead columbiformes, such as raptors and crows.[2] Falconers that keep these birds call the con-

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2-1 Mourning dove. Caseous nodules on either side of the trachea.
2-2 Mourning dove. Caseous nodules in area near the trachea.
2-3. Oropharnygeal region, Mourning dove. A mass is apparent within the oral cavity extending into the pharynx.

Gross photographs courtesy of Department of Pathology, National Zoological Park, 3001 Connecticut Ave. NW, Washington, D.C., 20008
http://nationalzoo.si.edu/default.cfm?ref=index.htm

dition "frounce". Additionally, the protozoan may be passed to a chick from the parental crop milk.[5]

At necropsy, the flagellates may be seen on a wet mount from carcasses that are up to 24 hours old.[6] A cytologic flagellum stain or silver stains on histologic preps may also elucidate the organism.

AFIP Diagnosis: Esophagus: Esophagitis, necrotizing, histiocytic and heterophilic, subacute, transmural, multifocal, severe with protozoa, mourning dove (*Zenaitha macroura*), avian.

Conference Comment: *Trichomonas gallinae* is a flagellated protozoan that contains four free anterior flagellae and one attached flagellum that trails along an undulating membrane, but does not extend beyond it.[3,8] In Columbiformes, transmission occurs directly through crop milk from infected adults to nestlings, through drinking water and food, as well as between adult birds during courtship behavior.[1,5] Transmission to raptors primarily occurs through ingestion of infected pigeons and doves.[2] *Trichomonas gallinae* has also been reported in Passeriformes (particularly canaries and zebra finches) and Psittaciformes (particularly budgerigars and cockatiels).[4]

Ultrastructural features of Trichomonads include piriform to spherical shapes, four anterior flagella of unequal size, well-developed undulating membrane, an axostyle, and a pelta surrounding the periflagellar canal.[8] The anterior flagellae emerge from the periflagellar canal which is reinforced by the pelta.[8]

Gross differentials for *Trichomonas gallinae* within the esophagus and crop include avian poxvirus, oral capillariasis, and candidiasis. Fibrinonecrotic lesions of wetpox, caused by avian poxvirus, are characterized by small white nodules to coalescing raised plaques with a diphtheritic membrane within the mouth, esophagus, trachea, pharynx, and larynx. Oral capillariasis produces almost identical gross lesions to that of *T. gallinae* and requires further testing to differentiate.[2] *Candida albicans* causes gray-white pseudomembranous patches in the mouth, pharynx, esophagus, and, most frequently, the crop.[7]

Contributor: Department of Pathology, National Zoological Park, 3001 Connecticut Ave. NW, Washington, D.C., 20008
http://nationalzoo.si.edu/default.cfm?ref=index.htm

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CASE III – 7-1907 (AFIP 3065931).

Signalment: Adult beaver, *Castor canadensis*

History: Adult male beaver found dead near a pond, March 2007.

Gross Pathology: The animal presented in poor body condition. Throughout the plantar aspect of the hindlimbs and lateral margins of the tail, there are multiple cutaneous ulcers. On incision of the abdominal wall, there is approximately 5 ml of clear serosanguinous fluid. There was moderate enlargement of the spleen and liver and throughout the parenchyma, there are focally disseminated pale yellow white nodules.

Laboratory Results: PCR for *Francisella tularemia* was negative. Aerobic culture yielded heavy growth of *Yersinia pseudotuberculosis* from the spleen and liver.

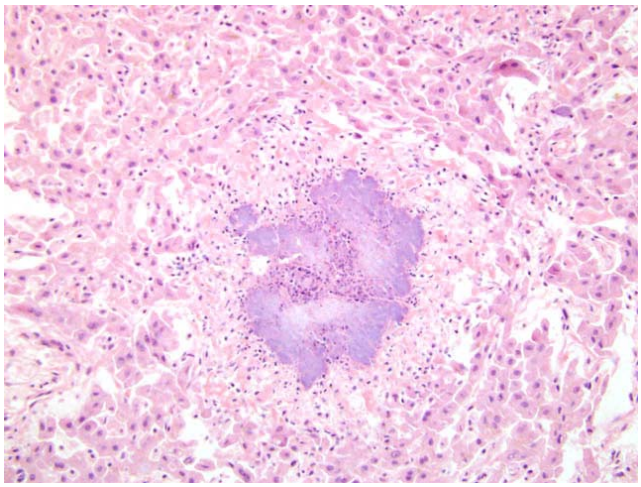
Histopathologic D escription: Immediately below the capsule and randomly throughout the parenchyma, there is multifocal to coalescing hepatocellular necrosis with lobulated colonies of coccobacilli frequently bound by variable neutrophilic infiltrates **(Fig. 3-1).**

Contributor's Morphologic Diagnoses:
Liver: Hepatitis, marked, necrotizing, multifocal to coalescing, acute, with florid lobulated colonies of extracellular coccobacilli

Contributor's Comment: The cause of death of this animal is attributed to the cumulative effects of the necrotizing hepatitis and splenitis and generalized emaciation. The heavy growth of *Yersinia pseudotuberculosis* from the liver and spleen was considered significant. Although *Y. pseudotuberculosis* is endemic worldwide, infections are more commonly recognized in temperate zones, particularly in Europe, and have been reported in a wide variety of domestic and wild mammals, including rodents, rabbits, deer, cattle, goats and sheep and birds, such as turkeys, ducks, geese, pigeons, pheasants and canaries. *Y. pseudotuberculosis* had previously been recovered in wildlife species between 1962 and 1973 (1 crow, 2 purple martens, 1 snowshoe hare, and 7 beavers) in Ontario, Canada and rat feces have been implicated as a source of human infection in Japan.[4,5] Exposure is predominantly by consumption of fecal contaminated food or water, or alternatively, ingestion of infected prey.[4] The epidemiology of infection is complex and not yet fully resolved; however, isolation of this bacterium from a beaver has important public health implications and the regional health authority has been notified and appropriate actions implemented. In humans, infection may result in mesenteric lymphadenitis, ulcerative ileitis, septicemia and erythema nodosum and most presenting patients are 4-15 year old males. From a wildlife population perspective, yersiniosis typically occurs in only select individuals in an area and, thus presumably has few implications for free ranging and marine mammals. In more densely populated communities, infection may be exacerbated by stress and reduced transmission distance.

AFIP Dia gnosis: Liver: Hepatitis, necrotizing, acute, random, multifocal to coalescing, severe, with large colonies of coccobacilli, beaver (*Castor canadensis*), rodent.

Conference Com ment: There are three species of *Yersinia* that are pathogenic for rodents and humans: *Y. pestis* (etiologic agent of bubonic and pneumonic plague), *Y. pseu dotuberculosis*, and *Y. en terocolitica*.[6] All pathogenic *Yersinia* spp. produce *Yersinia* Outer Proteins (Yop), which enable extracellular survival and proliferation **(Table 3-1).** A combination of various translocator, recognition, and effector Yops combine with a type III secretion system called Ysc to disarm macrophages and contribute to delaying the development of a cell-mediated immune response.[6]



3-1. Liver, beaver. Large colonies of coccobacilli within areas of hepatocellular necrosis. (H&E 200X).

Type III secretion systems are found within a wide variety of Gram-negative bacteria, and are used to deliver bacterial proteins into host cells.[7] It consists of a base structure that spans the inner and outer bacterial membranes and a needle-like structure that provides a conduit for the transfer of bacterial proteins into the host cell.[7]

There are 6 effector Yops, 5 of which are found in all three pathogenic *Yersinia* species: YopH, YopM, YopE, YpkA/YopO and YopJ/YopP. YopT is exclusively found in *Y. en terocolitica*.[3,7] YopB, YopD, and LcrV are required to translocate effector proteins into the host cell.[3,6] YopN and LcrG are part of a control and recognition system.[6]

Contributor: Animal Health Center, BC Ministry of Agriculture and Lands, Abbotsford, British Columbia

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***Table 3-1. Yersinia* Virulence Factors**

Type III secretion system	Composed of transmembrane base structure, and needle like conduit
PhoP	Plays a crucial role in ability to replicate within macrophages[3]
Translocation Yops	Required for translocation of Yops into the host cell, but is not required for excretion of Yops into extracellular space[3]
YopB	
YopD	
LcrV	
Control and Recognition Yops	Prevents Yop secretion prior to host cell attachement[2]
YopN	
LcrG	
Effector Yops	
YopH	Disrupts actin structures including focal adhesions and prevents phagocytosis by macrophages and neutrophils[3,7] , affects oxidative burst of macrophages and inhibits T- and B-cell signaling, and T-cell proliferation[6]
YopM	Scaffolding protein, only *Yersinia* effector that lacks catalytic activity[7]
YopE	Disrupts actin structures[6], prevent phagocytosis by macrophages and neutrophils[3]
YpkA/YopO	Disrupts actin skeleton, prevent phagocytosis by macrophages and neutrophils[3,7]
YopJ/YopP	Induces programmed cell death in macrophages, and functions as an acetyltransferase[7] resulting in inhibition of mitogen-activate protein kinase pathway[3]
YopT (*Y. enterocolitica*)	Inactivates RhoA[6], prevent phagocytosis by macrophages and neutrophils[3]

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CASE IV - 95-702 (AFIP 3070450).

Signalment: 2-year-old, female, Giant marine toad (*Bufo marinus*)

History: Found dead; no observable problems prior to death. Two cagemates have died of mycobacteriosis in the past.

Gross Pathologic Findings: Expanding the caudal portion of the base of the tongue and laryngeal/pharyngeal area are multiple, up to 5 mm cross sectional diameter, white to tan, caseous nodules that extend to the cranial portion of the left lung. There are multiple, up to 4 mm in diameter, white to tan, caseous nodules throughout the tissue of both lungs. The spleen is enlarged and measures 1.0 cm x 1.0 cm x 0.7 cm; there are multiple, up to 2 mm in diameter, caseous, white to tan nodules throughout the splenic parenchyma. The kidneys are uniformly dark red to purple and contain a few, small, up to 1 mm in diameter, white to gray foci, predominantly on the surface of the cranial portion of both kidneys.

Laboratory Results:
Lung cytology revealed acid fast bacilli.
Culture of the lung grew *Mycobacterium gordonae.*

Histopathologic Description: Lung: Multifocally expanding the intersitium are many granulomas that are up to 2 mm in diameter and are composed of a core of macrophages and few lymphocytes surrounded by a variably thick capsule of loose, fibrous connective tissue. Epithelium overlying these granulomas is often thinned and has lost the ciliated border. Occasionally, the epithelium is ulcerated. Between the faveolar walls are frequent infiltrates of macrophages, few lymphocytes and neutrophils and rare multinucleated giant cells. Rarely macrophages have peripheralized nuclei and peripheral cytoplasmic pallor.

Acid fast: There are scattered, positive bacilli throughout granulomas **(Fig. 4-1).**

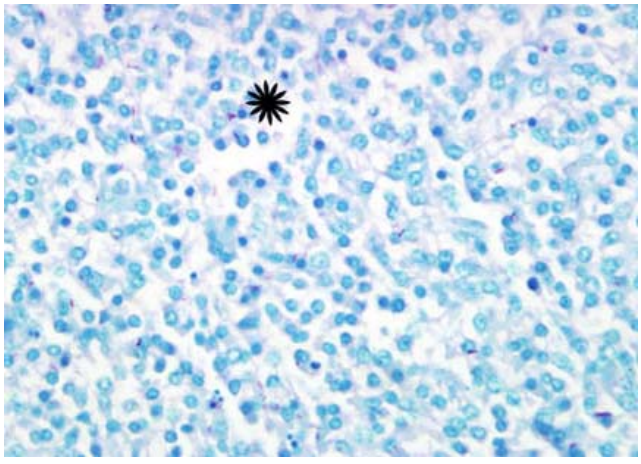
Contributor's Morphologic Diagnosis:
Lung: Pneumonia, granulomatous, chronic, multifocal, moderate, with intralesional acid fast bacilli consistent with *Mycobacterium* sp., giant marine toad (*Bufo marinus*)

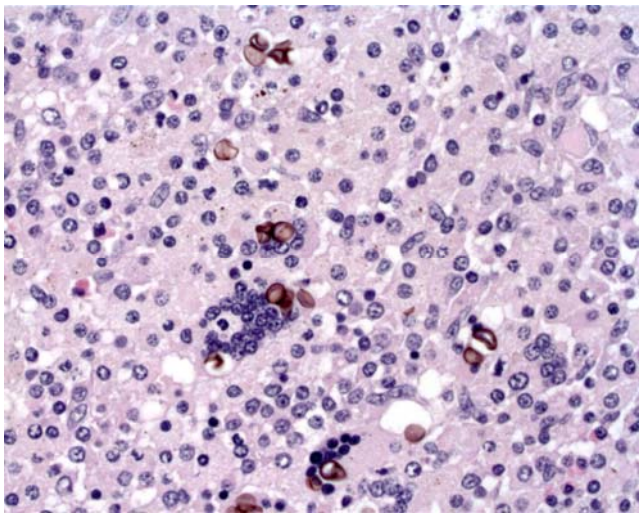
Contributor's Comment: Mycobacteriosis is common in animals and may be associated with a variety of pathogenic or environmental species. Historically, *Mycobacterium tuberculosis* and *M. leprae* most commonly affect humans, but *M. tuberculosis* has also been isolated from a number of non-human primates and elephants, and *M. leprae* may be enzootic in 9-banded armadillos.[5] *Mycobacterium gordonae* is commonly found in water and is not considered pathogenic.[5] However, compromise of the immune system can leave an individual open to opportunistic invasion by this organism. The toad in this case had systemic chromoblastomycosis which may have been associated with immunocompromise.

In amphibians, *Mycobacterium* induces granulomas similar to those precipitated by *M. tuberculosis* in humans. Key differences for amphibians include few multinucleated giant cells, a lack of mineralization, and a propensity for development in the skin.[4] Additionally, cytology and histology of amphibian lesions reveal numerous acid fast organisms, in contrast to the paucibacillary lesions of human TB. Cutaneous mycobacteriosis in the amphibian is most often associated with *M. marinum,* another waterborne mycobacterial species. The initial inflammatory response is disorganized and histiocytic and can be seen histologically by 2 weeks post-infection.[1] Mature granulomas composed of sheets of interdigitating epithelioid macrophages surrounded by a fine, fibrous capsule are seen by 8 weeks post-infection. Caseation of the core may occur in some granulomas[3] effectively walling off the bacteria from the host immune system. In this way, latent infection may be maintained.

AFIP Diagnosis: Lung: Pneumonia, granulomatous, multifocal, moderate, with acid fast bacilli consistent with *Mycobacterium* sp., giant marine toad (*Bufo marinus*), amphibian.

4-1. Lung, Giant Marine Toad. Scant positive bacilli scattered within granulomas (asterisk). (Acid fast 400X).





4-2. Lung, Giant Marine Toad. Multifocally, rarely within granulomas, there are few, 8-12 um, brown, thick-walled, dematiaceous (pigmented) fungal organisms (sclerotic bodies). They were not present in all sections. (H&E 400X)

Conference Com ment: Mycobacteria are non-motile, nonspore-forming, pleomorphic, acid-fast, weakly Gram-positive coccobacilli.[3] Their cell walls contain a large hydrophobic layer of mycolic acids which allows environmental and antimicrobial resistance. Increased amounts of trehalose dimycolate (cord factor) within the cell wall is also associated with increased virulence.[3] The *Mycobacterium* genus contains numerous strict pathogens (i.e. *M. tuberculosis* and *M. leprae*), as well as opportunistic pathogens (i.e. *M. avium* and *M. marinum*).[5] Tuberculosis is a term reserved for diseases caused by *M. tuberculosis* or *M. bovis* with all other conditions referred to as mycobacteriosis.[3]

The initiation of the cell-mediated immune response consists of activated macrophages secreting interleukin-12, which in turn skews the immune system to secrete interferon-g and interleukin-2 by CD4+ T-helper-1 lymphocytes.[3] Tumor necrosis factor-α and interferon-g act together to promote the formation of the tuberculoid granuloma.[3]

Granulomas in the amphibians induced by *Mycobacterium marinum* share many features with human tuburculosis granulomas.[1] They contain mature macrophages, epithelioid cells, and extracellular matrix components. Yet caseation does not appear to be a feature in frog granulomas even though it is seen in *M. marinum* infections of humans, goldfish and toads.[1]

Fungi consistent with a diagnosis of chromoblastomycosis **(Fig. 4-2)** were not present in all lung sections, so the entity was not discussed by the contributor. Chromoblastomycosis (chromomycosis) can be caused by a wide variety of brown pigmented, dematiaceous fungi, and infections have been seen in mammals and amphibians.[2] In amphibians the infection is often systemic with lesions in the skin, liver, lungs, and kidneys of stressed animals.[2]

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