Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2011-2012

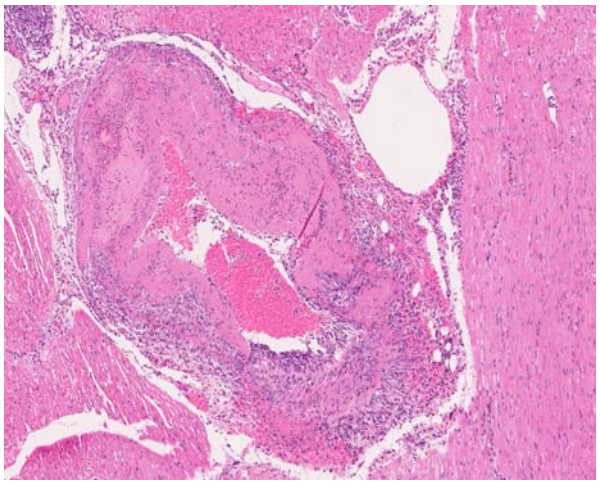
Conference 14

25 January 2012

CASE I: NADC MVP-2 (JPC 3065874).

Signalment: 5-month-old female white-tailed deer (*Odocoileus virginianus*).

History: Observed depressed, listless. Physical exam revealed fever (102.5 F), mild dehydration, normal auscultation of heart and lungs, no evidence of diarrhea. Treated with IV fluids, antibiotics and a non-steroidal anti-inflammatory drug. Deer died within 5 hours.



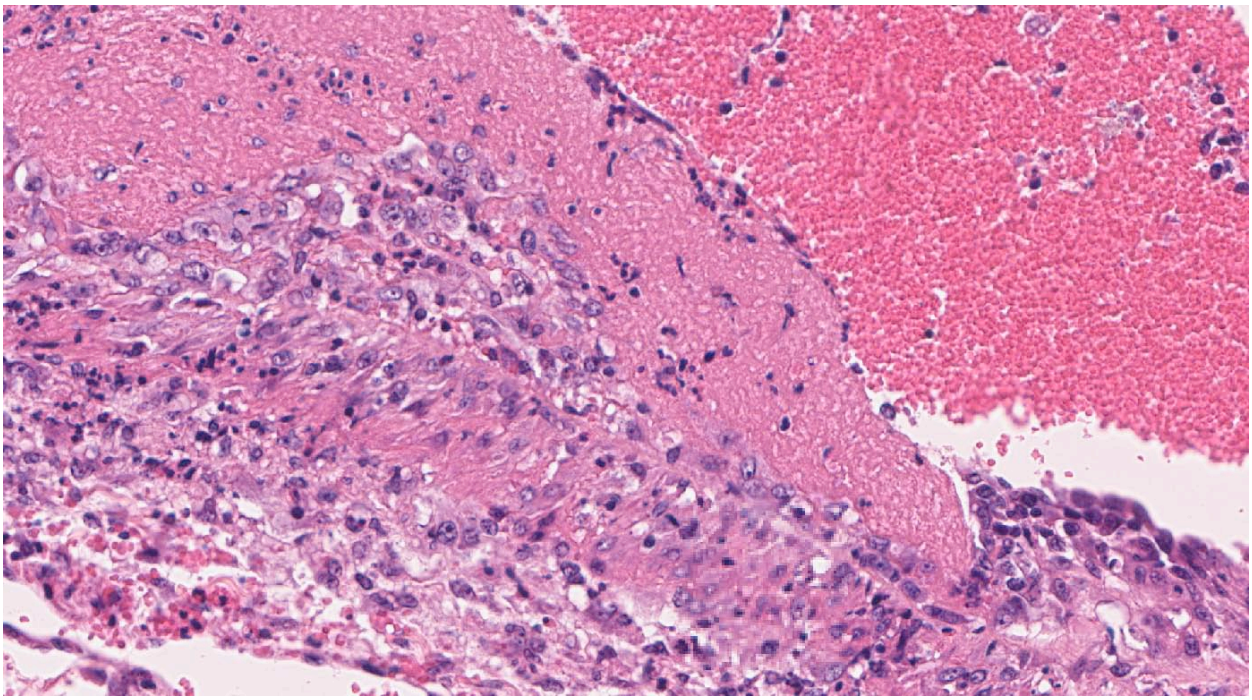
1-1. Heart, white-tailed deer. Necrotizing arteritis characterized by marked expansion of the wall by brightly eosinophilic protein, numerous inflammatory cells, and cellular debris (fibrinoid necrosis). (HE 67X)

Gross Pathology: The deer was of normal body condition with adequate deposits of body fat. There was crusty exudate around the eyes. Multifocal areas of hemorrhage were seen in the heart (epicardial and endocardial), lungs, kidney, adrenal glands, spleen, small and large intestines (mucosal and serosal surfaces) and along the mesenteric border, mesenteric lymph nodes and iliopsoas muscles. Multifocal ulcers were present in the pyloric region of the abomasum.

Laboratory Results:
PCR for OvHV-2: positive
PCR for EHV: negative
PCR for Bluetongue virus: negative
PCR for BVD: negative

Contributor's Histopathologic Description: Within the section of myocardium there is accentuation of medium to large arteries due to the infiltration of the vascular wall and perivascular spaces by inflammatory cells. Numerous lymphocytes and fewer neutrophils invade, and in some cases, efface the vessel wall. Fibrinoid degeneration and partially occluding fibrinocellular thrombi are present in the most severely affected vessels. Less affected vessels are characterized by large, rounded endothelial cells and intramural lymphocytes and neutrophils. Within the myocardium are multifocal areas of hemorrhage and scattered infiltrates of lymphocytes and macrophages.

Contributor's Morphologic Diagnosis: Myocardium: Arteritis and periarteritis, fibrinonecrotic,



1-2. Heart, white-tailed deer. Higher magnification of affected myocardial artery with effacement of the muscular wall by abundant subintimal proein, neutrophils, macrophages, and cellular debris (fibrinoid necrosis). (HE 172X)

lymphocytic, multifocal, acute, moderate to severe, with fibrinoid degeneration, thrombosis and myocardial hemorrhage, white-tailed deer (*Odocoileus virginianus*).

Contributor's Comment: Malignant catarrhal fever (MCF) is the clinical manifestation of the infection of certain ruminant species with one of a group of pathogenic gammaherpesviruses known as MCF viruses. Most domestic cattle and numerous exotic species of ruminants are susceptible to clinical disease that may be sporadic or occasionally epidemic in nature. Clinical disease can range from peracute to chronic and has been reported in various species of cervidae including white-tailed deer, black-tailed deer, mule deer, reindeer, muntjac deer, sika deer, Shira's moose, Pere David's deer, swamp deer, rusa deer, and red deer.[1,2,4,6,7,13,14,15] The disease is characterized primarily by lymphoproliferation, mucosal inflammation and vasculitis. Historically, 2 MCF viruses have been associated with clinical disease, one endemic in wildebeest, known as alcephaline herpesvirus –1 (AIHV-1), and the other endemic in sheep, ovine herpesvirus-2 (OvHV-2) known as sheep-associated MCF (SA-MCF). Only AIHV-1 has been propagated in vitro and partially characterized. OvHV-2 is the major MCF virus worldwide. Recently, however, 2 additional members of the MCF virus group have been associated with clinical disease in deer. An MCF virus of unknown origin that causes clinical disease in white-tailed deer[8], and an MCF virus endemic in goats, provisionally known as caprine herpesvirus-2 (CpHV-2) has been associated with chronic alopecia in sika and white-tailed deer.[5,6] The literature on MCF contains descriptions of various manifestations of disease with diverse organ involvement. The variable nature of disease expression is thought to result from multiple regulatory genes in gammaherpesviruses acquired during evolution. Cell type as well as host species may alter the expression of these genes.[6]

Deer infected with OvHV-2 can have a variety of clinical signs. Typically, the affected animal is lethargic, febrile with diarrhea that is often watery or contains blood. Death usually occurs within 48 hours. Animals that live longer may have excessive watery to mucous discharge from the eyes, mouth and nose. Mucosal erosions or ulceration may be present in the nose, oral cavity or anywhere in the gastrointestinal tract. Corneal opacity may lead to blindness in one or both eyes. Compared to cattle, MCF in deer is usually an acute disease with animals showing few clinical signs before death. Lesions are more hemorrhagic and involve the viscera of the gastrointestinal tract. MCF in cattle is less acute and the severity of visceral lesions is decreased. Clinical signs in cattle are variable. Grossly, MCF in cattle produces enlarged lymph nodes, corneal edema, cutaneous crusts and hyperemia, oral hyperemia and ulceration, mucopurulent nasal discharge, and erosive or ulcerative lesions throughout the digestive system.

Deer acquire the infection through direct or indirect

contact with the reservoir species, most commonly sheep. Reservoir species remain infected with the virus but do not show any clinical signs. Research suggests that deer with MCF do not transmit the virus to other deer. Sheep between the ages of 6 and 9 months of age shed much more virus than do sheep of other ages and therefore are considered most dangerous to deer and other susceptible species.[9,10] Nasal secretions are the predominant vehicle by which virus is spread from sheep to other species.[9,10] There is some evidence that MCF viruses may be spread by aerosol over significant distances. Research on MCF in bison has documented transmission over distances of 2.5 to 3 miles, against prevailing winds and in the absence of common water sources suggesting that some mechanisms of transmission remain undefined.

Recent research suggests that some susceptible species may be latently infected with virus and that there may be recrudescence of disease during periods of stress. Much remains unknown concerning latent infections in susceptible species. The incubation period (time from infection to the manifestation of clinical disease) is unclear and can be quite variable, ranging from a few days to several months. In some species clinical signs have been seen as late as 8 months after exposure to infected sheep. In the present case deer were housed on a pasture that was within 50 yards of a pasture containing sheep. Lambing had occurred on the pasture approximately 6 months earlier. Six-month-old lambs were still present on the sheep pasture.

Differential diagnosis for diseases that cause ulceration and necrosis of the oral and gastrointestinal mucosa and hemorrhage in deer and other ruminants include epizootic hemorrhagic disease in deer, bluetongue, bovine virus diarrhea-mucosal disease, rinderpest, and vesicular diseases. Important vesicular diseases to consider are foot and mouth disease and vesicular stomatitis, which are grossly indistinguishable from one another.

JPC Diagnosis: Heart: Arteritis, necrotizing and proliferative, multifocal, severe, with thrombosis and mild multifocal myocarditis.

Conference Comment: Histologically, malignant catarrhal fever (MCF) typically presents as perivascular and intramural infiltrates of lymphocytes and lymphoblasts with accompanying fibrinoid necrotizing vasculitis, which is unique to this disease. Conference participants noted that this particular case was difficult, because the mononuclear cell infiltrate was not easily identifiable as lymphoblastic. For this reason, polyarteritis nodosa, a noninfectious proliferative and necrotizing vasculitis which occurs sporadically in all species of domestic animals, was also considered in the differential diagnosis.[11]

The most common sites for vascular lesions are the brain and leptomeninges, carotid rete, kidney, liver, adrenal capsule and medulla, salivary gland, and any section of skin or alimentary tract with gross lesions. Lymphocytic infiltrates are also present in the kidneys, periportal areas of the liver, gastrointestinal mucosa, dermis, meninges, and heart. Microscopic lesions seen in other areas are an active proliferation of lymphoblasts in lymph nodes, especially in T cell-dependent areas of interfollicular and paracortical zones; and lymphocytic uveitis with exudate from ciliary processes into the filtration angle, resulting in corneal opacity of the eye.[3,12,17]

In MCF, the virus is usually associated with lymphocytes in adults, and primary viral replication occurs in small and medium-sized lymphocytes. T-lymphocyte proliferation is likely secondary to infection of large granular lymphocytes, which have T-suppressor cells and natural killer cell activity. Viral infection and dysfunction of these cells causes lymphoproliferation, T-suppressor cell dysfunction, and necrosis. Thevasculitis is presumed to be immune-mediated, but demonstration of immunoglobulin and complement components has been inconsistent.[16]

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References:

1. Beatson NS. Field observations of malignant catarrhal fever in red deer in New Zealand. *Biol of Deer Prod*. 1985;22:135-137.
2. Brown CC, Bloss LL. An epizootic of malignant catarrhal fever in a large captive herd of white-tailed deer (*Odocoileus virginianus*). *J Wildl Dis*. 1992;28(2): 301-305.
3. Gelberg HB. Alimentary system and the peritoneum, omentum, mesentery, and peritoneal cavity. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 4th ed. vol. 1. St. Louis, MO: Elsevier; 2012:1078, 1118.
4. Jessup DA. Malignant catarrhal fever in a free-ranging black-tailed deer (*Odocoileus hemionus columbianus*) in California. *J Wildl Dis*. 1985;21(2): 167-169.
5. Keel MK, Patterson JG, Noon TH, et al. Caprine herpesvirus-2 association with naturally occuring malignant catarrhal fever in captive sika deer (*Cervus nippon*). *J Vet Diagn Invest*. 2003;15:179-183.
6. Li H, Wunschmann A, Keller J, et al. Caprine hepesvirus-2-associated malignant catarrhal fever in white-tailed deer (*Odocoileus virginianus*). *J Vet Diagn Invest*. 2003;15:46-49.

7. Li H, Westover WC, Crawford TB. Sheep-associated malignant catarrhal fever in a petting zoo. *J Zoo Wildl Med*. 1999;30(3);408-412.
8. Li H, Dyer N, Keller J, et al. Newly recognized herpesvirus causing malignant catarrhal fever in white-tailed deer (*Odocoileus virginianus*). *J Clin Micro*. 2000;38(4):1313-1318.
9. Li H, Taus NS, Lewis GS, et al. Shedding of ovine herpsesvirus 2 in sheep nasal secretions: the predominant mode for transmission. *J Clin Microbiol*. 2004;42(12):5558-5564.
10. Li H, Hua Y, Snowder G, et al. Levels of ovine herpesvirus 2 DNA in nasal secretions and blood of sheep: implications for transmission. *Vet Microbiol*. 2001;79:301-310.
11. Maxie MG, Robinson WF. The cardiovascular system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5th ed. vol. 3, New York, NY: Elsevier Saunders; 2007:72-3.
12. Njaa BL, Wilcock BP. The ear and eye. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 4th ed. vol. 1. St. Louis, MO: Elsevier; 2012:1078, 1118.
13 Reid HW, Buxton D, McKelvey WA, et al. Malignant catarrhal fever in Pere David's deer. *Vet Rec*. 1987;121(12):276-277.
14. Tomkins NW, Jonsson NN, Young MP, et al. An outbreak of malignant catarrhal fever in young Rusa deer (*Cervus timorensis*). *Aust Vet J*. 1997;75(10): 722-723.
15. Williams ES, Thorne ET, Dawson HA. Malignant catarrhal fever in a Shira's moos (*Alces alces shirasi Nelson*). *J Wildl Dis*. 1984;20(3):230-232.
16. Zachary JF. Mechanisms of microbial infection. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 4th ed. vol. 1. St. Louis, MO: Elsevier; 2012:219.
17. Zachary JF. Nervous system. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 4th ed. vol. 1. St. Louis, MO: Elsevier; 2012:1078, 1118.

CASE II: 11-2389 (JPC 4002923).

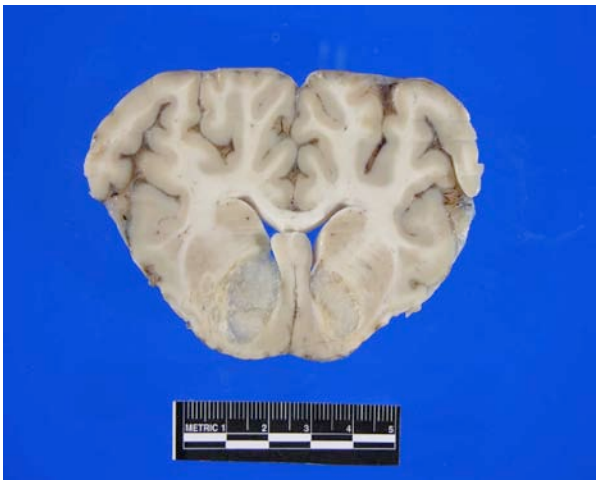
Signalment: Juvenile elk (*Cervus canadensis*), sex not specified.

History: The calf was seen circling for 2 days near Yakima, WA. The animal was shot and the head and liver were submitted to the Washington Animal Disease Diagnostic Laboratory for histopathology.

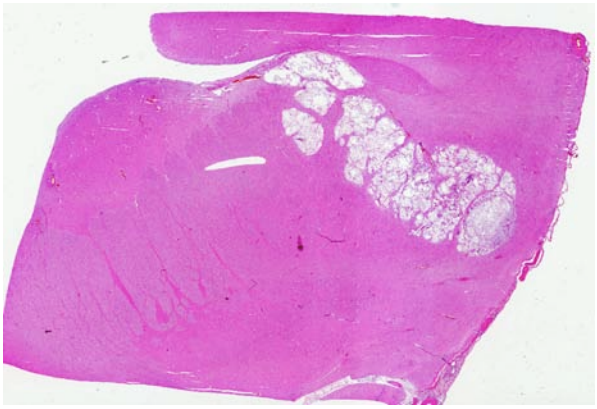
Gross Pathology: **Brain:** On cut surface just off of midline in the left rostral diencephalon and extending into the left caudal brain stem, there were numerous 0.5–2.0 cm diameter yellow gelatinous masses. There were no gross lesions in the tongue, retropharyngeal lymph nodes, mandibular lymph nodes, tonsils, pharynx, nasal cavity, tympanic bullae or liver.

Laboratory Results: After DNA extraction from the sample, the entire internal transcribed spacer (ITS) region of the ribosomal RNA gene (comprising ITS1, 5.8S, and ITS2) was amplified by PCR using universal fungal primers. When this PCR amplicon was directly sequenced, the sequence most closely matched that of *Cryptococcus gattii* (also called *C. bacillisporus*) (100% sequence identity with GenBank acc # EF081162 (ATCC strain 32609) and others) when compared with sequences in GenBank. The ITS signature sequence from this isolate exactly matched that of *C. gattii* ITS type 4, which corresponds with PCR-fingerprint molecular type VGII.

Contributor's Histopathologic Description: **Brain:** Disrupting the gray and white matter of the left aspect of the diencephalon, mesencephalon, and brain stem were multifocal to coalescing expansile inflammatory nodules composed of central areas of necrosis surrounded by epithelioid macrophages, fewer lymphocytes and plasma cells, and scattered multinucleated giant cells. Within these foci there were numerous 5–18 µm diameter, round extracellular yeasts with a 1 µm thin wall, narrow-based budding, and a 2–8 µm thick, amphophilic, mucicarmine-positive mucinous capsule. There was spongiosis of the adjacent neuropil with few infiltrating lymphocytes and plasma cells. Occasionally, there was expansion of the Virchow-Robin space by small numbers of lymphocytes, plasma cells and occasional macrophages (perivascular cuffing). The leptomeninges overlying the cerebrum and brainstem were expanded by moderate numbers of lymphocytes, plasma cells, and macrophages associated with the same yeast bodies.



2-1. Cerebrum, calf. On cut surface just off of midline in the left rostral diencephalon and extending into the left caudal brain stem, there are numerous 0.5–2.0 cm diameter yellow gelatinous masses. Photograph courtesy of the Department of Veterinary Microbiology and Pathology and the Washington Animal Disease Diagnostic Laboratory, Pullman, WA 99164-7040 www.vetmed.wsu.edu



2-2. Cerebrum, calf. Focally extensive area of necrosis within the diencephalon containing numerous dimorphic yeasts and minimal inflammation. (HE 63X)

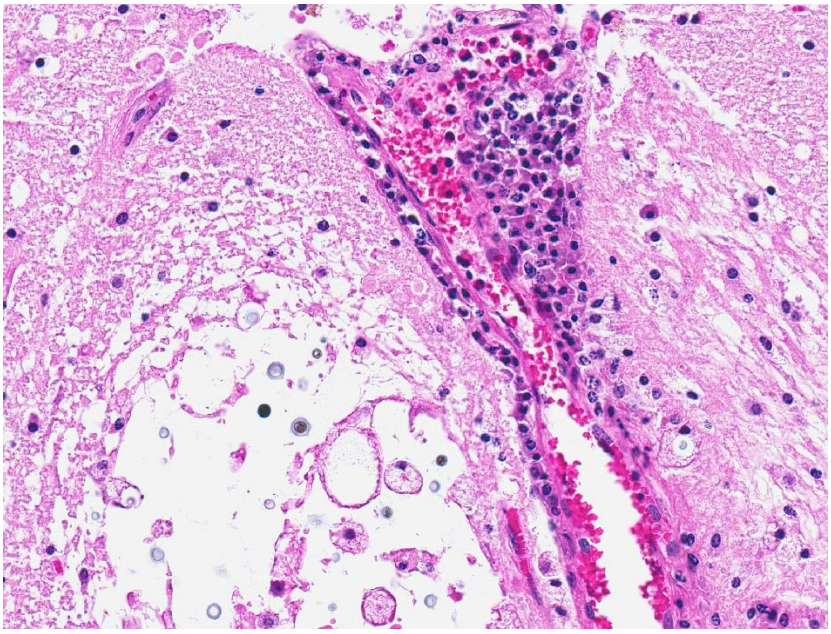
Sections of liver (not submitted) had mild centrilobular fatty degeneration of hepatocytes. No lesions were identified in other structures of the head.

Contributor's Morphologic Diagnosis: Meningoencephalitis, granulomatous, multifocal to coalescing, moderate, with many intralesional carminophilic yeasts (*Cryptococcus* sp.).

Contributor's Comment: Cryptococcosis is a localized to systemic fungal disease with worldwide distribution caused by a basidiomycete yeast-like fungus.[4] The two main species associated with disease in humans and animals are *Cryptococcus neoformans* and *C. gattii*, which vary in distribution, ecology and pathogenicity. Reproduction occurs primarily asexually, but the organism does possess the capability for sexual reproduction. Histologic lesions in animals caused by the two species are identical; differentiation must be done either by serotyping or at the molecular level.[1] Capsular serotypes B and C represent *C. gattii*; serotype A is *C. neoformans* var *grubii* and serotype D is *C. neoformans* var *neoformans*. Recently,

multilocus sequence typing (MLST) has separated *C. gattii* into 4 lineages, VGI – VGIV.[2]

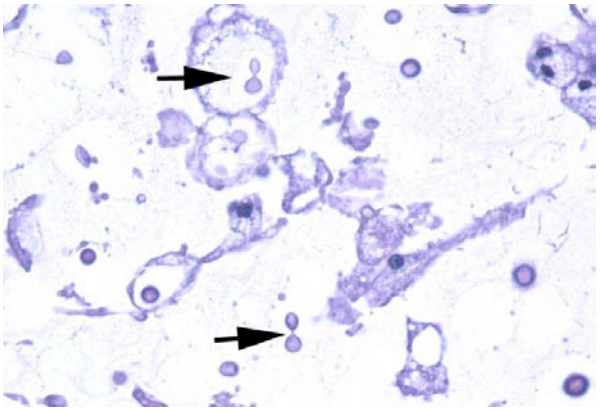
Cryptococcal disease is neither contagious nor zoonotic; animals and people acquire the infection by inhaling infectious particles from the environment. *C. neoformans* is found in soil and bird droppings, especially from pigeons.[4] *C. gattii* is found in association with eucalyptus trees and recently in the coastal Douglas fir and western hemlock forests of British Columbia and the Pacific Northwest US.[6] The infectious particle is likely the basidiospore, although dehydrated yeast bodies may also be infectious.[1] *C. neoformans* has emerged as an important secondary infection in people with HIV/AIDS; while *C. gattii* also causes disease in immunocompromised hosts, cryptococcosis in previously healthy individuals is more likely to be caused by *C. gattii*. Cryptococcosis in cats, the most common veterinary species affected, is rarely related to documented immunodeficiency.[4] Respiratory infections are the most common manifestation of disease, although systemic spread, especially to the brain, is not unusual. Some reports suggest that, in people, *C. gattii* infection is more likely to result in neurologic disease than is infection by *C. neoformans*.[1,5]



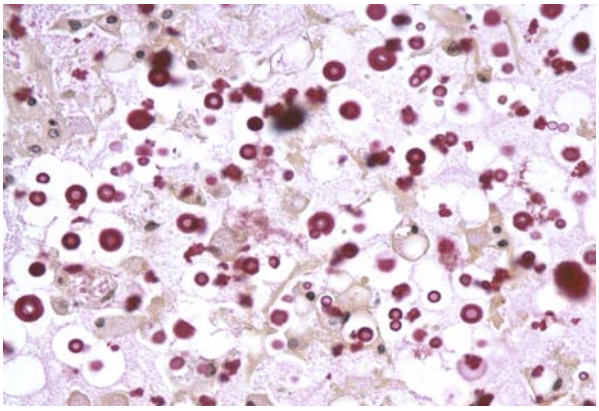
2-3. Cerebrum, calf. Higher magnification of necrotic area containing numerous 8-15µm dimorphic yeasts with a 2µm amphophilic cell wall. The yeasts are surrounded by a clear capsule. Within the area of necrosis, there are small numbers of large macrophages, some of which have engulfed yeasts. There is mild spongiosis of the adjacent neuropil and the lymphoplasmacytic cuffing of vessels. (HE 140X)

Cryptococcal virulence factors probably evolved as protection against ingestion by environmental amoeba; mammalian infection is likely accidental.[1] Those virulence factors include the ability to grow at temperatures above 30° C and the distinctive large, polysaccharide capsule which protects against phagocytosis and killing by neutrophils. Production of melanin by both pathogenic species protects the organisms against oxidative damage.

Although originally thought to be restricted to the eucalyptus forests of Australia, Southeast Asia and other tropical regions, *C. gattii* was identified in animal cases of cryptococcosis on Vancouver Island in 2000 and subsequently spread to people and animals in British Columbia, Washington and Oregon.[2] Retrospective studies suggested that *C. gattii* may have circulated in Southern California for much longer. The mechanism of the switch from tropical to temperate



2-4. Cerebrum, calf. Cryptococcus gatti, exhibiting characteristic narrow-based budding (HE 320X)



2-5. Cerebrum calf. Carminophilic 2-8 µm capsule surrounding C.gatti. (HE 320X).

climates is unknown. However, most eucalyptus associated outbreaks in Australia are of molecular type VGI, whereas 90% of isolates from the Pacific Northwest are type VGIIa, and southern California isolates are type VGIII, the genotype commonly identified in Mexico. This suggests that different genotypes have different biogeoclimatic distributions.[5]

This case of neurologic cryptococcosis caused by *C. gattii* in a juvenile elk in Yakima County, WA represents further evidence of the spread of *C. gattii* infection within the Pacific Northwest region of the United States. A previous report of a case or cases of *C. gattii* infection in Yakima County provides no details.[5] There is limited information on *C. gattii* infection in wildlife. In 2006, a culture survey of wildlife species on Vancouver Island and lower mainland British Columbia found a 2% positive rate in nasal cavity swabs, a rate similar to that of sampled domesticated species.[6] Although no similar survey of wildlife in the Pacific Northwest is published, we would expect additional cases of *C. gattii* disease in wildlife in our area in the future.

JPC Diagnosis: Brain, dienchepalon: Meningoencephalitis, necrotizing and histiocytic, multifocal, severe, with numerous encapsulated yeasts, etiology consistent with *Cryptococcus gattii*.

Conference Comment: Conference participants discussed four important virulence factors, mentioned by the contributor. First is the mucopolysaccharide capsule, which prevents phagocytosis by alveolar macrophages, confers resistance to opsonization, impairs phagocytosis and leukocyte migration, activates complement and suppresses T-cell responses. Secondly, phenoloxidase, a laccase, produces the antioxidant melanin from diphenolic compounds, protecting the yeast from oxidative damage. Thirdly, serine protease cleaves fibronectin and other basement membrane proteins and may aid in tissue invasion. Fourth is the ability to grow at 37° C, a temperature at polysaccharide capsule expression is enhanced. Rabbits are naturally resistant to cryptococcosis, due to their higher normal body temperature of 39.5° C, which inhibits fungal replication and dissemination.[3]

Urease expression by *Cryptococcus* spp. appears to promote sequestration in microcapillaries, which is a critical step in dissemination to the brain. The organism's predilection for the central nervous system may be partially attributed to the lack of alternative pathway complement components in cerebrospinal fluid, which would otherwise bind to the carbohydrate capsule and enhance phagocytosis and killing by neutrophils.[3]

Protective immunity against *Cryptococcus* spp. requires a Th1 pattern of cytokine response, to include interleukin-12 (IL-12), IL-18, interferon gamma, tumor necrosis factor beta, granulocyte-macrophage colony stimulating factor, macrophage inflammatory protein-1, and monocyte chemoattractant protein-1. Humoral factors, such as opsonization by antibody and complement are important in clearance.[3]

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References:

1. Bovers M, Hagen F, Bockhout T. Diversity of *Cryptococcus neoformans-Cryptococcus gattii* species complex. *Rev Iberoam Micol*. 2008;25:S4-S12.
2. Byrnes III, EJ, Marr KA. The outbreak of *Cryptococcus gattii* in western North America: Epidemiology and clinical issues. *Curr Infect Dis Rep*. 2011;13:256-261.
3. Carroll SF, Guillot L, Qureshi ST. Mammalian model hosts of cryptococcal infection. *Comp Med*. 2007;57(1):9-17.
4. Caswell JL. Williams KJ. The respiratory system In: Maxie, MG ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5th ed. Edinburgh, Scotland: Elsevier; 2007;642-643.
5. Datta K, Bartlett KH, Baer R, et al. Spread of *Cryptococcus gattii* into the Pacific Northwest region of the United States. *Emerging Infect Dis*. 2009;15:1185-1191.
6. Duncan C, Schwantje H, Stephen C, et al. *Cryptococcus gattii* in wildlife of Vancouver Island, Bristish, Columbia, Canada. *J Wildlife Dis*. 2006;42:175-178.

CASE III: 53364 AFIP (JPC 4002986).

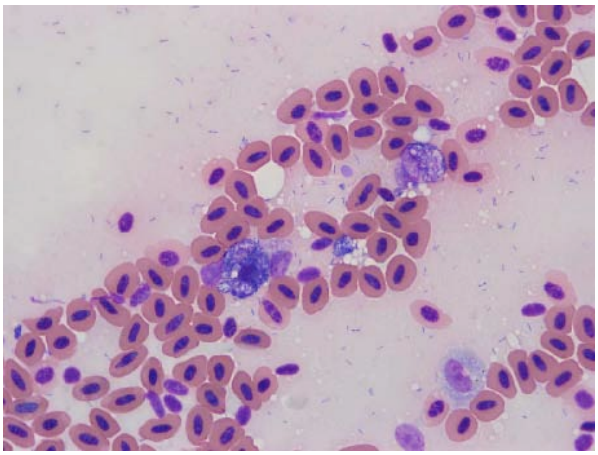
Signalment: African jacana bird (*Actophilornis africanus)*, female 12+ years old, 251 g, wild- caught long-term captive.

History: A keeper found this bird dead near a stream in its exhibit. It had been moved from a nearby exhibit onto the current exhibit approximately 40 days prior. Following the move it had been eating well, and was approaching the keepers and taking food items at each feed-out.

Gross Pathology: At necropsy, this jacana was moderately autolyzed, and had adequate adipose stores and pectoral muscle mass. Multiple enlarged, firm interphalangeal joints were present on both feet. The nail was absent from the right first digit and the distal aspect was swollen to greater than 1 cm in diameter. The dorsolateral lung fields were mottled dark red. Approximately 50 petechial hemorrhages were present in the subepicardium of the heart and subserosal surface of the proventriculus. The spleen was diffusely swollen, soft, and dark pink-gray. Impression smears of the lung, spleen, and intestine yielded mixed inflammatory cells and large numbers of gram-positive rod bacteria which were often within inflammatory cells, particularly histiocytes.

Laboratory Results: Bacterial cultures of postmortem kidney samples preserved at -70 C yielded 3+ growth of *Erysipelothrix rhusiopathiae*, and 1+ growth each of *Escherichia coli* and *Enterococcus sp*.

Contributor's Histopathologic Description:
Kidney: Multifocally throughout the sections glomerular capillaries and small-caliber, thin-walled intertubular vessels are expanded by dense aggregates of tightly packed dark blue bacterial colonies which occasionally completely occlude vessel lumina. In these areas, the bacterial colonies are often observed to be contained within the cytoplasm of large irregular cells with eccentrically displaced nuclei. Medium and occasionally larger caliber veins and smaller arterioles contain moderately increased numbers of large blue-gray histiocytic cells. In some vessels these cells form small aggregates or dense sheets and contain small to large clusters of dark blue rod bacteria similar to those observed in the capillaries. In the most severely affected areas of some sections, occasional individual proximal tubules are lined by dissociated epithelial cells with hypereosinophilic hyalinized to clumped cytoplasm and pyknotic to fragmented nuclei (necrosis). Multifocally collecting ducts are mildly to markedly dilated and contain pale blue granular to fibrillar material admixed with amorphous basophilic debris. Low numbers of lymphocytes, heterophils, plasma cells and histiocytes are scattered throughout



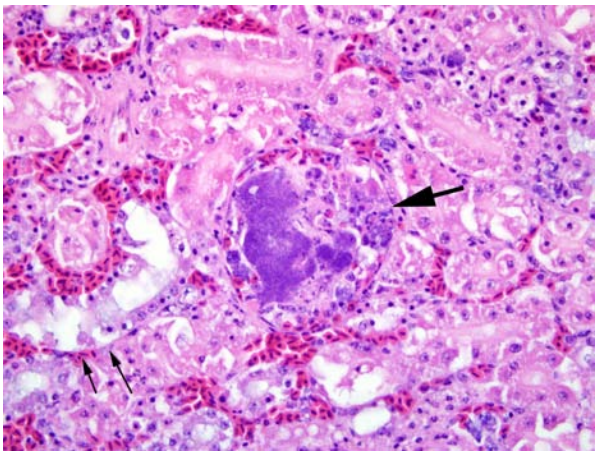
3-1. Lung, African jacana. Cytologic preparation showing several histiocytes distended by numerous phagocytosed bacilli. (Wright-Giemsa 1000X)

the sections. Rarely, random individual tubules are mineralized, and infrequently dark orange-brown granular material is present in the cytoplasm of tubular epithelium. The intracellular bacteria were strongly gram-positive with Goodpasture's Gram stain.

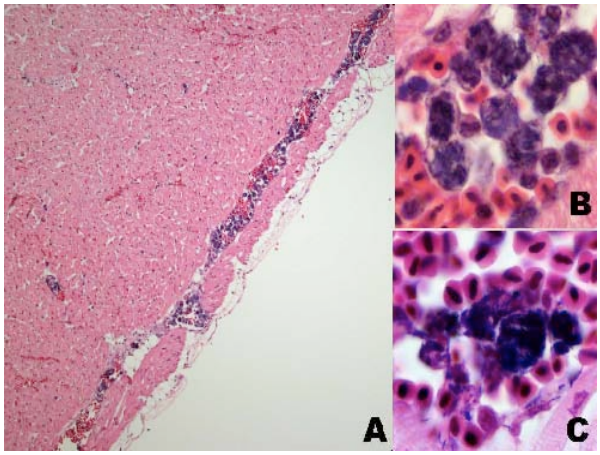
Other tissues: Large intracytoplasmic aggregates of bacteria similar to those in the kidney were present in phagocytic cells within vessels of most tissues. These were associated with hemorrhage and necrosis in the heart, spleen, and intestine. Histiocytosis was especially prominent in the spleen and was accompanied by a remarkably large number of intracellular bacteria. Multiple fibrinocellular thrombi were also present in the lung. Proliferative and ulcerative pododermatitis (bumblefoot) was confirmed and gram-positive and gram-negative rod bacteria, gram-positive coccoid bacteria and superficial fungal hyphae were present in the ulcerated areas. Supplemental digital images include a composite of affected heart in which a vessel contains histiocytes with intracytoplasmic gram-positive rod bacterial colonies.

Contributor's Morphologic Diagnosis: 1. Kidney: intravascular histiocytosis with intracellular gram-positive rod bacteria.
2. Kidney: mild to moderate multifocal acute tubular necrosis, mild multifocal granulocytic and lymphocytic interstitial nephritis, and moderate multifocal collecting duct ectasia with urate accumulation (not present in all sections).

Contributor's Comment: The bacterium *Erysipelothrix rhusiopathiae* is a cosmopolitan gram-positive, non-spore-forming, facultative anaerobe with numerous serotypes and variable virulence.[1,8,9] It has been documented to cause severe disease in a wide variety of animals including domestic and wild birds,



3.2. Kidney, African jacana. Necrotic glomerulus exhibiting a large intravascular bacterial embolus. There is a large aggregate of polymerized fibrin and necrotic debris within Bowman's capsule (large arrow). To the left of the glomerulus(small arrows), there is a tubule whose epithelium exhibits epithelial necrosis. (HE 400X)



3.3 Heart, African jacana. Numerous vessels contain histiocytes distended by numerous phagocytosed bacilli (A – 100X, B- 400X.) The bacilli stain blue-black (gram-positive) on a Goodpasture's gram stain. (Gram, 1000X)

mammals ranging from whales to mice, and occasional reptiles. It is also a common commensal on aquatic and marine fishes and invertebrates.

Infection of livestock with *E. rhusiopathiae* results in significant financial losses, particularly in swine and turkey production units. *E. rhusiopathiae* is considered normal pharyngeal flora in up to 50% of swine and bacteria also cause significant disease, most commonly septicemia, polyarthritis, and endocarditis.[8] The septicemic form in swine often leads to disseminated intravascular coagulation with classic cutaneous manifestations characterized by diamond-shaped pink to purple raised foci commonly referred to as "diamond skin disease."[8] It causes a rapidly fatal septicemia in turkeys and other birds with inapparent to generalized petechiation in multiple organs and fibrinopurulent exudate on organ surfaces and joints.[1] In many cases there are limited histologic lesions, with colonies of intravascular bacteria with little or no inflammation as the primary feature.[3,11] While it may cause sporadic disease in a variety of wildlife, it is notable that *E. rhusiopathiae* has caused multiple significant mass mortality events involving hundreds of wild eared grebes in Nevada and brown pelicans in California, nearly an entire group of released captive raised kakapo in New Zealand, and one of 60 remaining Hawaiian 'Alala crows.[3,4,5,10]

Due to the ubiquitous nature of the bacteria, the potential for mass mortality events in birds, and the ability to infect individual birds of high value, *E. rhusiopathiae* should be considered as a potential cause of sudden death or sepsis in birds in zoological collections. The jacana in this case was wild caught in Tanzania and retained in the collection for approximately 12 years in multiple enclosures, most recently with a long-term mate, and had successfully raised several clutches of chicks. She had a history of proliferative pododermatitis lesions dating back to approximately 1 year after entering the collection. At necropsy the digital articular and skin lesions were chronic and proliferative, but also ulcerated and heavily colonized with opportunistic fungi and mixed bacteria. It hypothesized that infection in this case occurred via the skin wounds. An outbreak of *E. rhusiopathiae* affecting more than 300 chuckars was attributed to foot trauma caused by relocation from enclosures with solid flooring to wire-bottomed flooring.[2] Exposure to bedding material previously used by swine was the suspected source of the *E. rhusiopathiae* in the chukars. Disease in the jacana was suspected to have resulted from contamination of plantar skin wounds by bacteria in soil or enclosure bedding, water, mud, or other aquatic source associated with the stream. Bacteriologic surveys for potential sources were not evaluated in this case however, and dietary or other alternative sources including rodents or stray wildlife could not be ruled out.

JPC Diagnosis: Kidney: Glomerulitis and nephritis, necrotizing and histiocytic, multifocal, moderate, with numerous intravascular bacterial colonies.

Conference Comment: The degree of autolysis present in the sections hampers thorough interpretation of some of the pathologic tubular changes in this interesting case. Erysipelothricosis in birds often has such an acute course that the animal dies before many appreciable pathologic lesions occur.[7]

Erysipelothrix rhusiopathiae adheres to cells by producing neuraminidase, an enzyme that cleaves alpha glycosidic linkages in neuraminic acid, a reactive mucopolysaccharide in cell membranes. *Erysipelothrix rhusiopathiae* has hemagglutinating

activity that can result in complement-dependent hemolysis in severe acute disease. The hemagglutinating activity is believed due to the high neuraminidase activity in virulent strains.[6,10]

Acute to subacute systemic erysipelothricosis causes generalized septicemia and endothelial swelling of capillaries and venules, leading to adherence of monocytes to vascular walls and thus vascular fibrinoid necrosis, fibrin thrombosis, and invasion of vascular endothelium by bacteria. Chronic erysipelothricosis leads to polyarthritis, synovitis, fibrosis and articular cartilage destruction. Vegetative valvular endocarditis frequently occurs, resulting in vasculitis, myocardial infarcts, destruction of valve endocardium, and splenic and renal infarcts.[6,10]

Erysipelothrix rhusiopathiae is on a short list of gram positive bacilli important in veterinary medicine; others include *Actinomyces* spp., which are filamentous, *Corynebacterium* spp., and *Listeria monocytogenes*.

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References:

1. Bricker JM, Saif YM. Erysipelas. In: Calnek BW, ed. *Diseases of poultry*. 10th ed. Ames, IA: Iowa State University Press; 1997;302–313.
2. Butcher G, Panigrahy B. An Outbreak of Erysipelas in Chukars. *Avian Dis*. 1985;29(3):843-845.
3. Franson JC, Galbreath EJ, Wiemeyer SN, et al. Erysipelothrix rhusiopathiae Infection in a captive Bald Eagle (*Haliaeetus leucocephalus*). *J Zoo Wildl Med.* 1994;25(3):446-448.
4. Gartrell BD, Alley MR, Mack H, et al. Erysipelas in the critically endangered kakapo (*Strigops habroptilus*). *Avian Pathol.* 2005;34(5):383-7.
5. Jensen WI, Cotter SE. An outbreak of erysipelas in eared grebes (*Podiceps nigricollis). J Wildl Dis.* 1976;12(4):583-586.
6. Maxie MG, Robinson WF. Cardiovascular system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 5th ed. vol. 3. San Diego, CA: Academic Press; 2007;27-29.
7. Mazaheri A, Lierz M, Hafez HM. Investigations on the pathogenicity of Erysipelothrix rhusiopathiae in laying hens. *Avian Diseases*. 2005;49(4):574-576.
8. Qinning Wang Q, Chang B, Riley T. Review: Erysipelothrix rhusiopathiae *Vet Micro.* 2010;140:405–417.
9. Shibatani M, Suzuki T, Chujo M, et al. Disseminated intravascular coagulation in chickens inoculated with Erysipelothrix rhusiopathiae. *J Comp Pathol*. 1997;117(2):147-156.
10. Thompson K. Bones and joints. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 5th ed. vol. 1. San Diego, CA: Academic Press; 2007;163-164.
11. Work TM, A Donna Ball, B, Mark Wolcott. Erysipelas in a Free-ranging Hawaiian Crow (*Corvus hawaiiensis*) *Avian Dis.* 1999;43:338-341.

CASE IV: SP-10-6530 (JPC 4002892).

Signalment: 12-year-old male ring-tailed lemur (*Lemur catta*).

History: This captive ring-tailed lemur was housed in a Midwestern American zoo and first presented with labored breathing and was immobilized for a physical examination. It had significant respiratory effort under anesthesia. A large mass was observed on a thoracic radiograph, obscuring the heart shadow. The lemur recovered from anesthesia but continued to have labored breathing and lethargy. It was humanely euthanized.

Gross Pathology: A 9 x 7 x 9 cm gelatinous mass was present in the pleural cavity, replacing and displacing most normal lung parenchyma.

Laboratory Results: None performed.

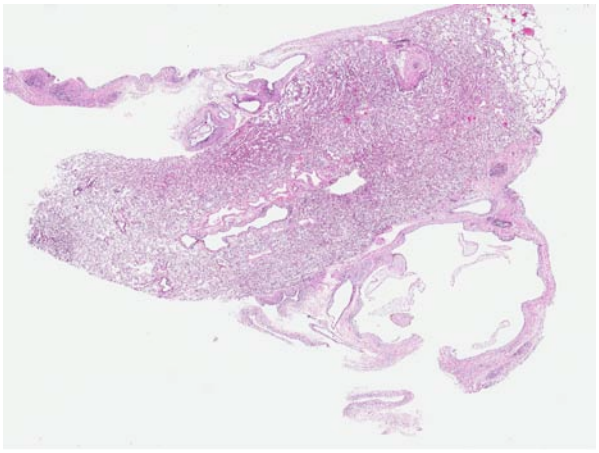
Contributor's Histopathologic Description: The lung tissue is largely replaced in many sections by large thick-walled fibrous cavities with adjacent and intramural abundant lymphoplasmacytic nodular aggregates of macrophages, neutrophils, and eosinophils. The cavities are often lined by activated macrophages. Within many cavities are large, often multiple, viable and degenerate larval cestodes characterized by their segmented integument, calcareous corpuscles, a scolex with a rostellum bearing hooklets, and spinous tegument. Exogenous budding from the end opposite, the scolex is present in some slides. In the remaining lung, there were multifocal areas of parenchymal collapse of airways, peripheral emphysema, and pools of neutrophils, fibrin, and hemosiderin-laden macrophages in alveolar spaces and within capillaries. Many megakaryocytes were also circulating in the capillaries. Small nodular areas of lymphoplasmacytic inflammation are noted on the serosa of the intestine and on the diaphragm.

Contributor's Morphologic Diagnosis: Lung: Severe granulomatous and fibrous pleuropneumonia with intralesional larval cestodes (presumptive *Cysticercus longicollis,* the larval form of *Taenia crassiceps).*

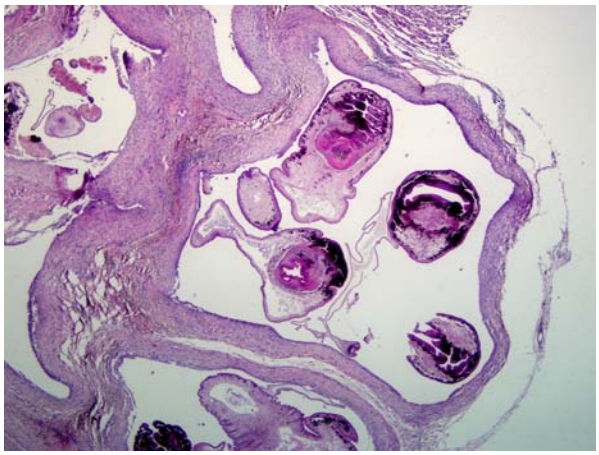
Contributor's Comment: Larval cestodes often present as cysts, of which there are several forms: cysticercoids, cysticercus, coenurus, and hydatid cyst.[3] Of these, the presented case is a cysticercus based on the presence of only one scolex in each bladder (though bladder walls have degenerated in many of the fibrous cavities). Other key features to note are the thick tegument and the deeply basophilic calcareous corpuscles which are most numerous on the neck and scolex, but not in the bladder wall.[3]

Pulmonary, pleural, or peritoneal infection of lemurs by cysticerci has been well documented anecdotally and in the literature.[2,4] Based on these reports, one of which included molecular confirmation, and the similar morphology including rare budding seen in this case, the most likely etiology is *Cysticercus longicollis,* the larval form of *Taenia crassiceps,* though other *Taenia* sp. are possible.

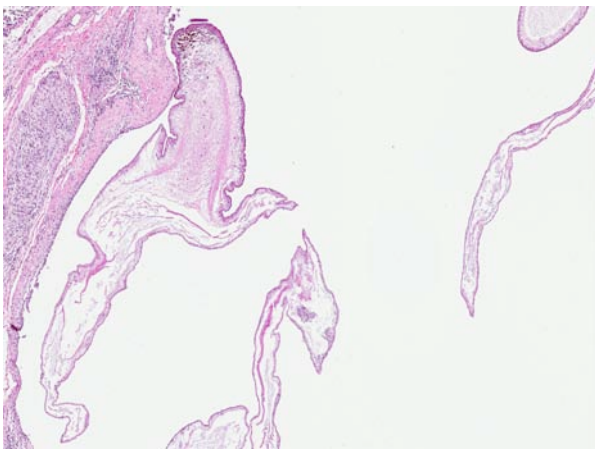
Exposure to feces of the definitive host (typically wild canids, but also domestic cats and dogs) or consumption of infected secondary rodent hosts are possible modes of transmission to the lemur in this case. After ingestion by an aberrant host, it is not well-documented as to how the cysticerci migrate to the peritoneal or pleural cavities, but once there, these cases can be remarkably fulminating since the organism can proliferate by budding both endogenously and exogenously. Infection and rapid proliferation is associated particularly with



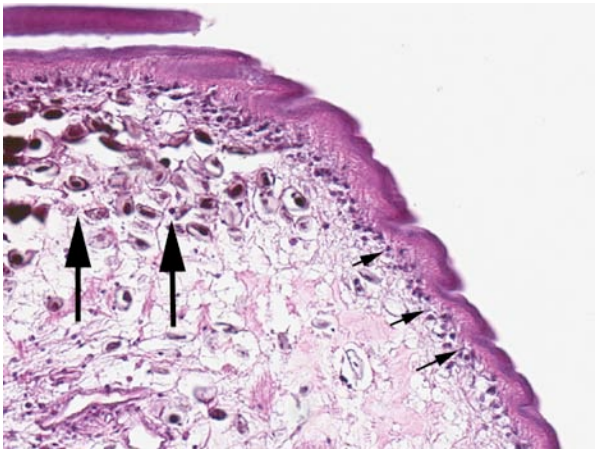
4-1. Lung, ring-tailed lemur. Lung parenchyma is replaced (lower left) by a large fibrous cyst contain cross sections of several cysticerci. (HE, 40X)



4-2. Lung, ring-tailed lemur. Within one of the fibrous cysts, there are several degenerating (mineralizing) cysticerci showing the scolex and invaginations of the thin bladder wall. (HE, 100X)



4-3. Lung, ring-tailed lemur. Cysticercus demonstrating evaginated scolex and bladder. (HE, 200X)



4.4. Lung, ring-tailed lemur. Tangential section through cysticercus demonstrating the serrated cuticle, spongy body cavity, somatic cell nuclei (small arrows), and numerous calcareous corpuscles (large arrows). (HE 320X)

immunosuppression. This disease is zoonotic, most recently associated with AIDS patients. There was no history or clinical pathology data to suggest this lemur is immunocompromised, but the perceived high frequency of cysticercosis in lemurs might indicate a predisposition in this exotic species which has presumably never been exposed to canid or felid parasites in their evolutionary history until very recently.

JPC Diagnosis: Lung: Cysticerci, multiple, with fibrosis and mild histiocytic and eosinophilic pneumonia.

Conference Comment: There is some variation among slides, and some participants may have received two sections of lung in which the histiocytic and eosinophilic pneumonia and bronchitis are a more prominent feature due to the presence of larval cysticerci in airways.

Other important cysticerci in veterinary species are included in the following chart[1]:

ADULT TAPEWORM	DEFINITIVE HOST	LARVAL FORM	INTERMEDIATE HOST	SITE
Taenia saginata	man	*Cysticercus bovis*	cattle	muscle
Taenia solium	man	*Cysticercus cellulosae*	pig, man	muscle
Taenia (*Multiceps*) *multiceps*	dog	*Coenurus cerebralis*	sheep, cattle	CNS
Taenia hydatigena	dog	*Cysticercus tenuicollis*	sheep, cattle, pig	peritoneum
Taenia ovis	dog	*Cysticercus ovis*	sheep	muscle
Taenia pisiformis	dog	*Cysticercus pisiformis*	rabbit	peritoneum, liver
Taenia serialis	dog	*Coenurus serialis*	rabbit	connective tissue
Taenia taeniaeformis	cat	*Cysticercus fasciolaris* (strobilocercus)	mouse, rat	liver
Taenia krabbei	dog	*Cysticercus tarandi*	reindeer	muscle
Taenia mustelae	wild felids		rodents	liver
Diphyllobothrium latum	bear, man		fish	muscle
Diphyllobothrium pacificum	seal, sea lion		marine birds	muscle

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References:

1. Bowman DD, Lynn RC, Eberhard ML. *Georgi's Parasitology for Veterinarian*. 8th ed. St. Louis, MO: Saunders; 2003:130-153, 269, 275, 375-7.
2. Dyer NW, Greve JH. Severe Cysticercus longicollis cysticercosis in a black lemur (*Eulemur macaco macaco*). *J Vet Diagn Invest.* 1998;10:362-364.

3. Gardiner CH, Poynton SL. *An Atlas of Metazoan Parasites in Animal Tissues.* Washington, DC: Armed Forces Institute of Pathology; 2006.
4. Luzon M, de la Fuente-Lopez C, Martinez-Nevado E, et al. *Taenia crassiceps* cysticercosis in a ring-tailed lemur (*Lemur cata*). *J Zoo Wildl Med*. 2010;41:327-330.