Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 22

15 April 2020

CASE I: NO-16-1279 (JPC 4099471).

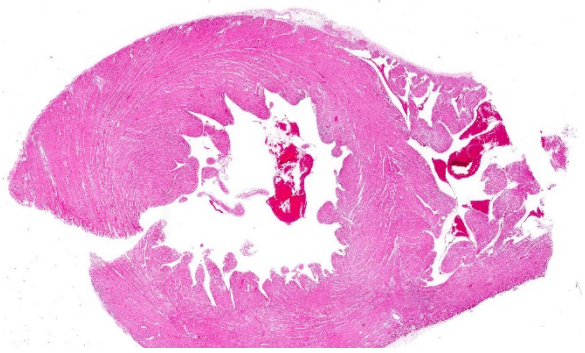
Signalment: Adult male white-headed capuchin (*Cebus capucinus*).

History: Several New World and Old World monkeys (white-headed capuchin (*Cebus capucinus*), booted macaque (*Macaca ochreata*), and sooty mangabey (*Cercocebus atys*)) in this primate facility had a sudden onset of severe depression, fatigue, lethargy, and sleepiness, then died within a few hours after first clinical signs.

Gross Pathology: A 3.45 Kg adult male white-headed capuchin (*Cebus capucinus*) was in fair nutritional condition (body condition score of 4/9). Scleral and oral mucosa was diffusely pale pink. The heart was moderately enlarged and veins were dilated with clotted blood. There were multiple foci of slightly pale discoloration scattered throughout the left ventricular wall and near the heart apex. The discolored pale tan areas extended approximately 5-7 mm into the underlying myocardium. The apical region of pallor was approximately 3 mm in diameter at the epicardial surface. Lung lobes were diffusely pink; however, the right middle lobe had multifocal distinctly dull red demarcated, depressed areas.

The liver was slightly enlarged; the tip of the right lateral liver lobe extended 5-10 mm caudal to the costal margin.

Throughout the cerebrum and meninges, vessels were dilated and prominent. All other organs were grossly unremarkable.



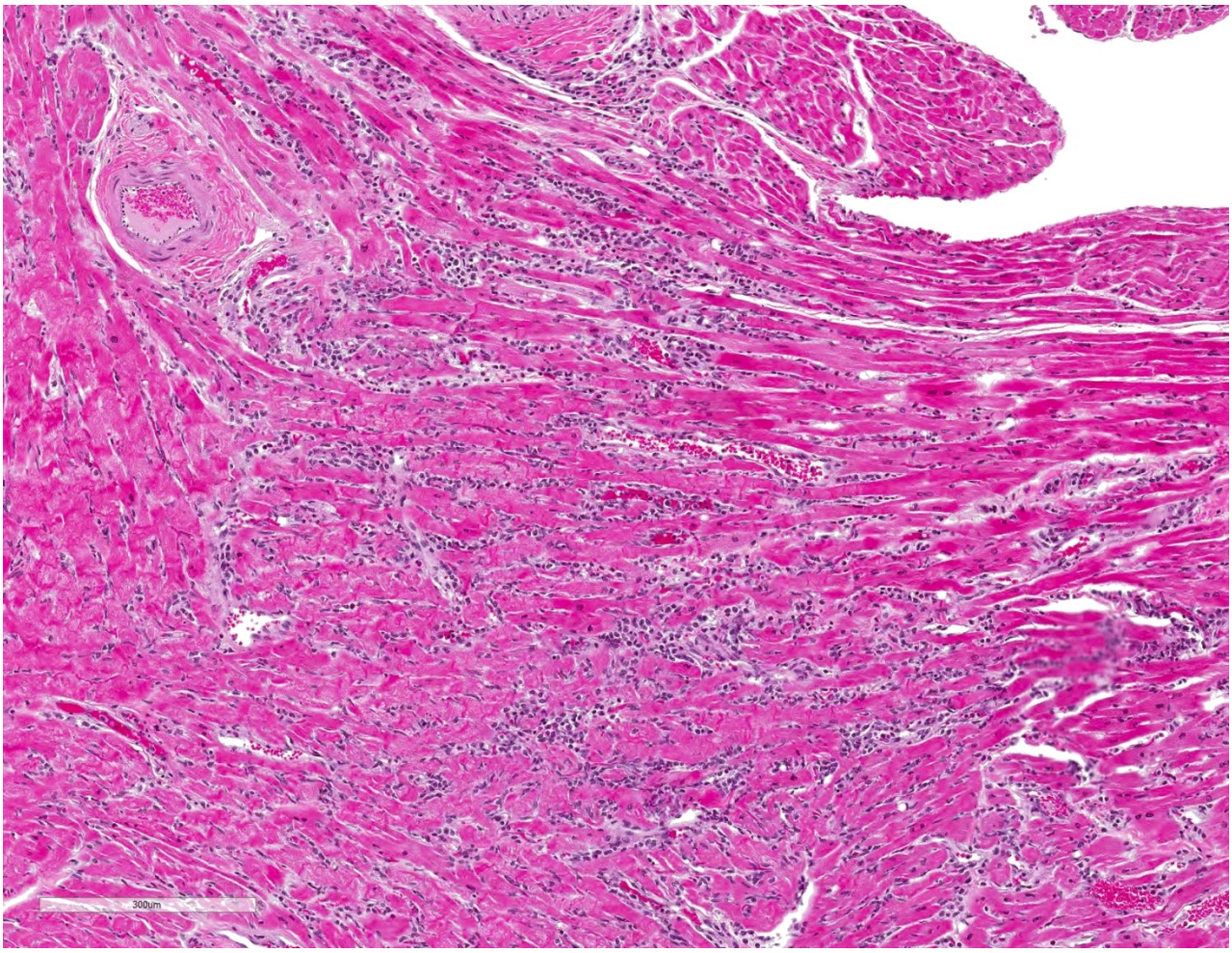
Heart, capuchin monkey. A cross section of the left tentacle and tip of left atrium is submitted for examination. There are no lesions at subgross examination. (HE, 5X)

Laboratory results:

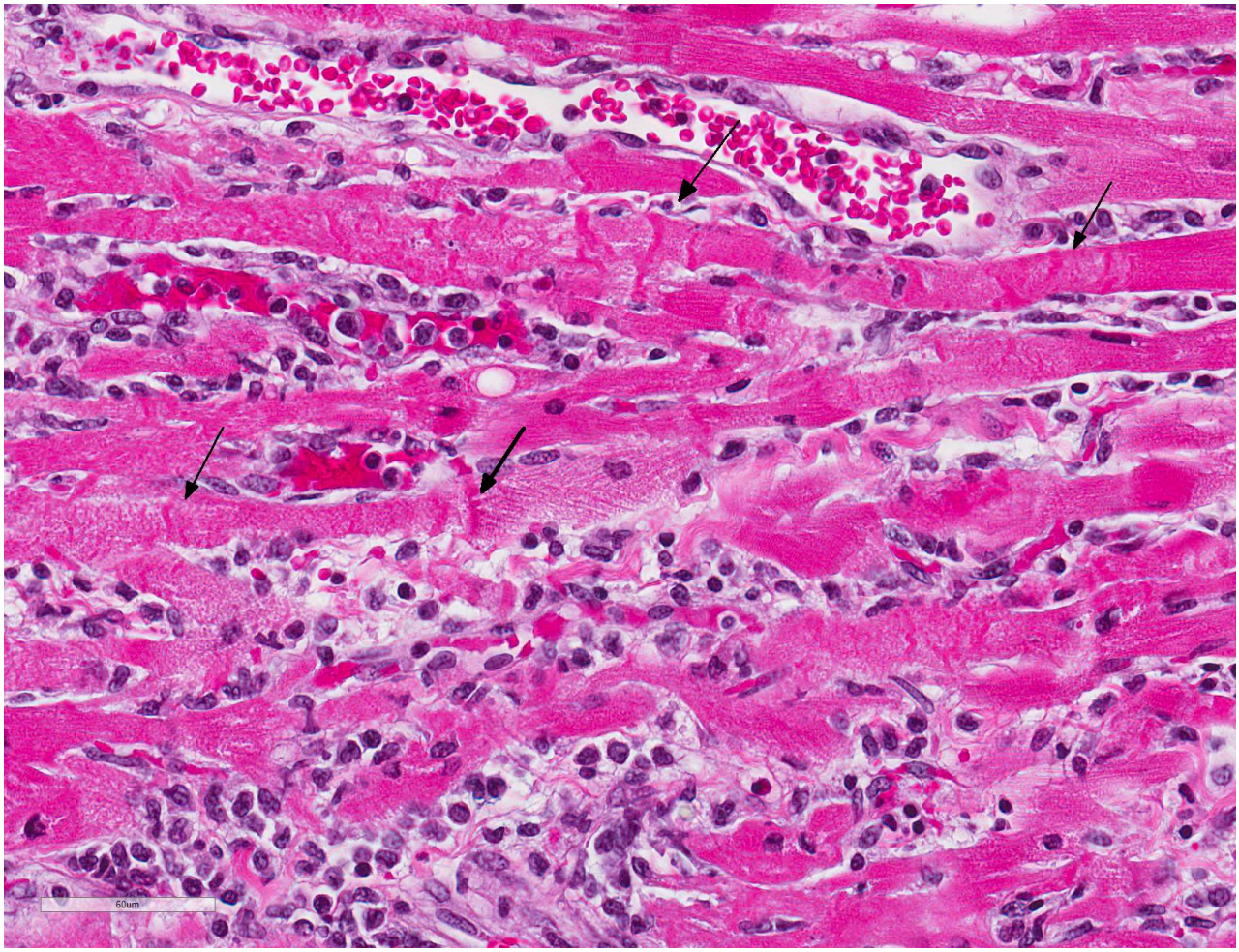
- Bacteriology: Rare colonies of *Staphylococcus aureus* were isolated from submitted pulmonary tissue, and no bacterial colonies were isolated from submitted hepatic tissue.

- Virology: Virus isolation demonstrated prominent cytopathic effects from submitted fresh frozen pooled specimens including heart and brain. Encephalomyocarditis virus was detected from cell culture supernatant by PCR and confirmed by genetic sequencing. PCR to detect Herpesvirus, West Nile virus, and *Francisella tularensis* were negative.

Microscopic Description: All representative sections of the heart contain similar inflammatory processes and will be described collectively; however, the degree of severity varies between sections. Multifocally, within examined sections of left and right cardiac ventricles, there are variably dense interstitial infiltrates of



Heart, capuchin monkey. Multifocally and randomly, the interstitium is markedly expanded by a cellular infiltrate. (HE, 100X)



Heart, capuchin monkey. In areas of inflammation, moderate numbers of lymphocytes and macrophages expand the interstitium. Cardiac myocytes demonstrate necrotic chantes including shrinkage, fragmentation contraction band formation (black arrows), pyknosis andkaryorrhexis. (HE, 400X)

mixed inflammatory cells dissecting around the myocardial fibers and surrounding occasional vessels in some areas. These leukocytes include lymphocytes, plasma cells, and histiocytes mixed with fewer neutrophils. The affected cardiomyocytes are variably degenerating or necrotic characterized by hypereosinophilia, loss of cellular detail, formation of contraction bands, and nuclear pyknosis and karyorrhexis. In some areas, there is intracytoplasmic and extracellular basophilic granular mineralization.

Contributor's Morphologic Diagnosis:

Heart: Myocarditis, necrotizing, moderate to severe, multifocal to coalescing

Contributor's Comment:

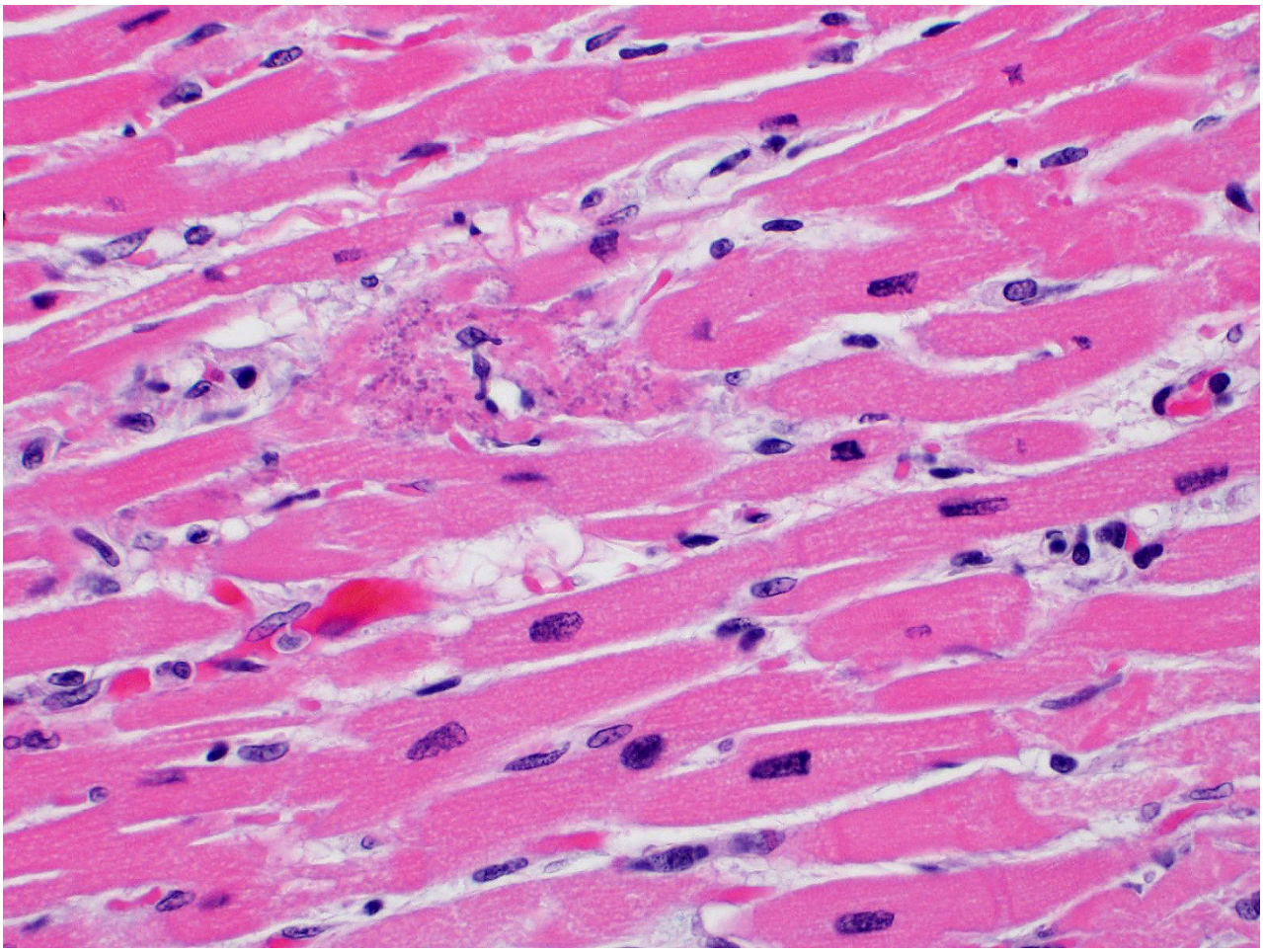
Encephalomyocarditis virus (EMCV) is a small non-enveloped single-stranded RNA virus, classified as a member of family *Picornaviridae*, genus *Cardiovirus*. The virus is known to cause myocarditis and encephalitis in a variety of domestic mammals including pigs and several zoo and wildlife species including non-human primates.[1,3,5,6,12] EMCV infection is also

sporadically reported in humans, although the vast majority of cases are mild and asymptomatic.[10]

Rodents are considered to be reservoir hosts for EMCV in some studies,[1,5] though studies have associated EMCV inoculation with severe to fatal myocarditis and necrotizing pancreatitis in Mongolian gerbils (*Meriones unguiculatus*) as well as acute orchitis in Syrian hamsters (*Mesocricetus auratus*).[7,8]

Infected non-human primates have a rapid disease course including severe depression and lethargy progressing to acute heart failure and death within 24 hours of clinical presentation. In some cases, the affected animal may suffer from progressive respiratory distress prior to death.[2, 5] As in this case, gross findings are typically limited to the heart as evidenced by pale white discoloration or variable petechial and ecchymotic hemorrhage.[1,2,5]

Hydropericardium, hydrothorax, pulmonary congestion, and ascites may be observed in some cases. [2,3] The principal histologic findings of EMCV-infected non-human primates include multifocal regions of myocardial degeneration and necrosis with associated variable degrees of lymphocytic to mixed interstitial inflammatory infiltrates.



Heart, capuchin monkey. Necrotic myofibers are occasionally stippled with granular mineral. (HE, 400X)

Neural lesions are uncommon in non-human primates, which suggests a cardiotropic nature of the virus in these hosts;[4] however, some studies showed neurotropism of this virus in naturally and experimentally infected animals.[1,6] Other infectious diseases that can be associated with myocarditis in non-human primates include West Nile virus, *Trypanosoma cruzi*, and *Borrelia burgdorferi*.

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JPC Diagnosis: Heart: Myocarditis, necrotizing and lymphocytic, multifocal, random, mild to moderate, with mild lymphocytic epicarditis and endocarditis.

JPC Comment: EMCV was first identified in a captive gibbon in 1945 that died suddenly from pulmonary edema and myocarditis. Mice inoculated with edema fluid from the affected monkey developed myocarditis and hindlimb paralysis. A similar virus was isolated 4 years later in the Mengo district of Entebbe, Uganda from a paralyzed rhesus macaque which was shown to be antigenically indistinct via sero-neutralization, but both viruses were considered distinct from the Theiler's murine encephalomyelitis virus (another cardiovirus).[2] The first outbreak in swine was noted in Panama in 1958.[2]

Although mice are considered to be the natural reservoir, rats are often implicated in transmission to other species, including non-human primates and swine. Transmission is most likely the result of fecal contamination of feeds, although horizontal transmission has been identified in swine and suspected in non-human primates.[11]

This virus has been shown to infect a wide range of non-human primates, including rhesus macaques, mandrills, chimpanzees, marmosets, owl monkeys and squirrel monkeys. [11] There have been a number of significant outbreaks in captive baboons, suggesting that this species may be uniquely susceptible.[11] Many infected primates are found dead with minimal premonitory signs, or suffer from rapidly progressing biventricular heart failure with prominent edema and froth or fluid from the nose and mouth. As mentioned by the contributor, the clinical picture in non-human primates is primarily that of cardiac infection, however, neurotropic strains may also cause lymphocytic infiltrates in the cerebrum.[11]

Encephalomyocarditis virus will also cause cardiovascular disease in a number of other species including pygmy hippopotamuses and exotic hoofstock, lemurs, tapirs, rhinoceroses, and elephants[9] (perhaps explaining the elephant's mythic fear of the mouse.) Transmission within zoo collections is considered the result of fecal and infected carcass contamination of feed. Myocarditis and heart failure is always present; neurologic lesions are uncommon.[9]

While considered a zoonosis, disease in humans is mild and somewhat controversial. Human cell lines are readily infected. EMCV has been incriminated in a number of cases of febrile human illness with clinical signs of headache, nausea and malaise, when EMCV virus was detected using molecular techniques in the absence of other viruses. Seropositivity to EMCV has ranged from 3-15% in the general population with higher rates in humans with occupational exposure and hunters. [2]

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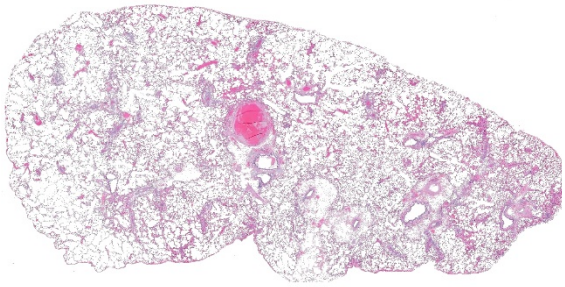
CASE II: Case 2 (DVD) (JPC 4136179).

Signalment: 2.9 year old male rhesus macaque (*Macaca mulatta*)

History: The animal was assigned to a study investigating the effects of co-stimulatory blockade on delaying rejection of renal porcine xenotransplants. The animal presented with acute severe dyspnea one-month post-transplantation.



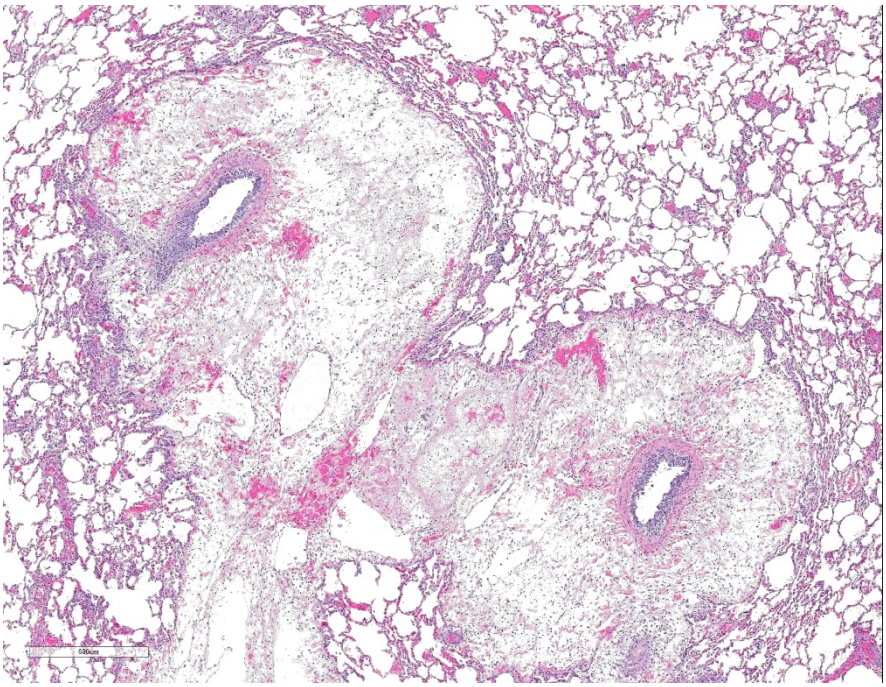
Lung, rhesus macaque. At subgross examination, pulmonary arterioles are highlight by marked edema, vessels throughout the section are outlined by a cellular infiltrate, and there is a large focal central pulmonary thrombus. (HE, 6X)

Gross Pathology: Red-tinged foam and liquid was present surrounding the nares and muzzle. Similar foam was present within the larynx and trachea. There was approximately 5mL of straw-colored fluid within the pleural cavity with few floating fibrin aggregates. The lungs were wet, heavy, failed to collapse, and were mottled red-pink.

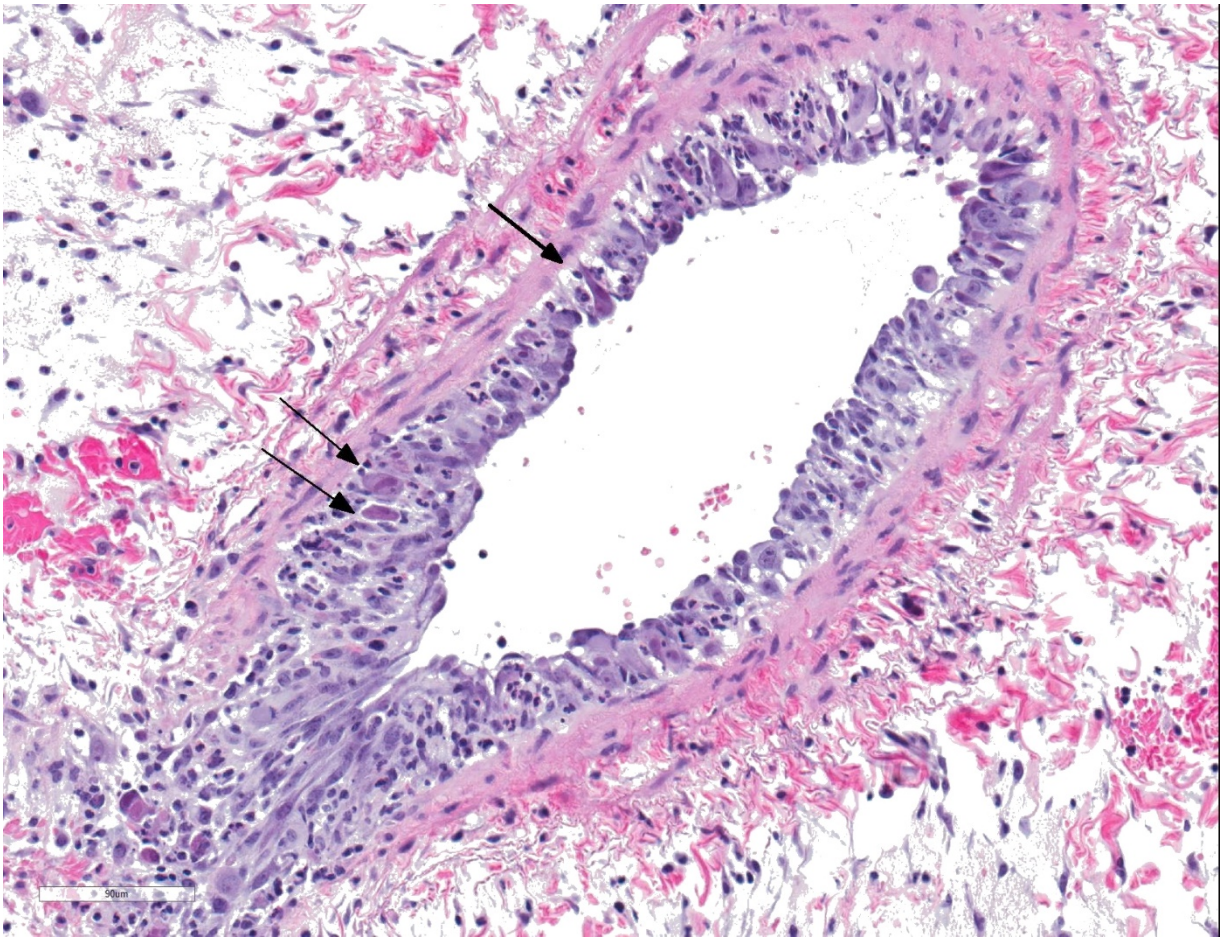
Laboratory results:

Hematologic and serum biochemical analyses demonstrated a leukocytosis ($11x10^3$) with mild neutrophilia, lymphopenia, monocytosis, mild anemia, mild hypoproteinemia with low-normal albumin, mild azotemia, hyperphosphatemia, hyperglycemia, and hyperkalemia.

Microscopic Description: Lungs: There are numerous small and medium caliber blood vessels that are lined by endothelial cells with markedly enlarged nuclei and cytoplasm (cytomegaly) and occasional Cowdry type-A eosinophilic intranuclear inclusion bodies. Affected vessels are frequently infiltrated by neutrophils, lymphocytes, and histiocytes within the tunica media and intima (vasculitis). Multiple vessels are surround by abundant clear space (perivascular edema) and lesser amount of free erythrocytes (hemorrhage). Few larger vessels have abundant proliferation of the tunica intima with infiltration by neutrophils There are scattered coalescing regions where alveolar spaces contain free erythrocytes, fibrin, neutrophils, necrotic debris, and increased numbers of alveolar macrophages that frequently contain abundant eosinophilic



Lung, rhesus macaque. Higher magnification of the marked edema which expands arteriolar adventitia and compresses adjacent alveoli. Lymphatics are markedly expanded and there is multifocal hemorrhage. (HE, 38X)



Lung, pulmonary arteriole, rhesus macaque. There is marked hyperplasia and hypertrophy of arteriolar endothelium and several hypertrophic endothelial cells contain large oblong intranuclear viral inclusions (arrows). The intima and inner media is expanded by edema, infiltrating neutrophils, and cellular debris. (HE, 240X)

cytoplasm (alveolar edema). There are multiple patchy regions where alveolar septae are expanded by similar mixed inflammatory cells. There are rare intranuclear inclusion bodies present within histiocytes and type-1 pneumocytes.

Contributor's Morphologic Diagnosis:

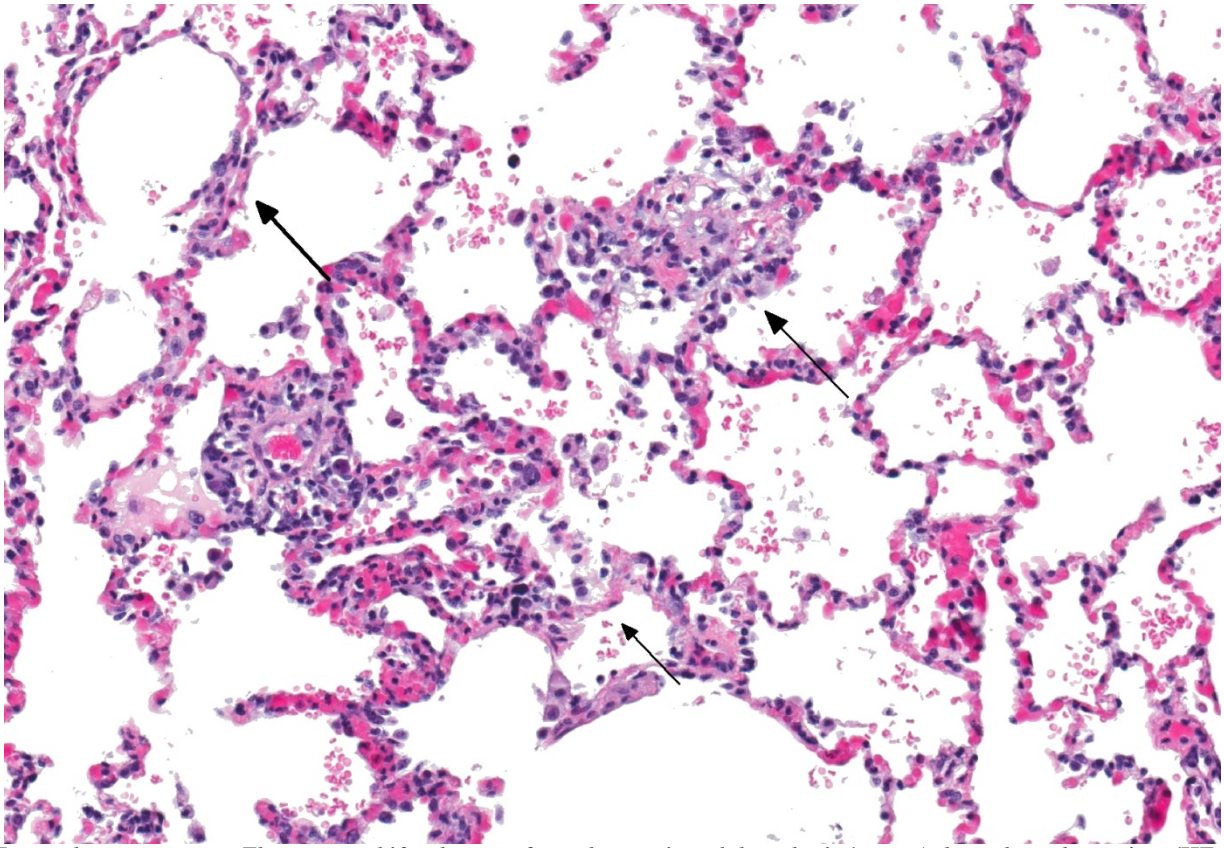
Lungs: Severe, multifocal widespread, neutrophilic and lymphocytic small and medium artery vasculitis with endothelial cytomegaly, Cowdry type-A intranclear inclusions (cytomegalovirus) and perivascular edema; Mild to moderate, multifocal to coalescing, neutrophilic and lymphocytic interstitial pneumonia with alveolar edema and hemorrhage.

Contributor's Comment: Rhesus cytomegalovirus (*macacine herpesvirus 3*), is a double-stranded DNA virus in the family betaherpesvirus that is commonly identified in non-specific pathogen free colonies of rhesus macaques, with 50% of infants seropositive by 6 months of age and almost 100% by 1 year old.[22] Transmission is believed to be horizontal, as vertical transmission is exceedingly rare, which mimics the human virus[4]. Infections are usually asymptomatic with the virus

remaining latent until an immunosuppressive event occurs, allowing for recrudescence.

Specifically, T-cells play an important role in controlling CMV replication,[16] which are also the target of many immunosuppressive therapies in organ transplantation and in SIV and type-D retroviral infections.[1] Cytomegaloviral infection/recrudescence has been reported as a complication leading to death or euthanasia in multiple nonhuman primate species undergoing solid organ or stem cell transplantation, including rhesus[13,15] and cynomolgus[12] macaques and baboons.[2]

Although it is difficult to assess the complication rates in nonhuman primate transplant studies due to the relatively small numbers of animals reported in the literature, the rate of post-transplantation infections is thought to be 14%.[10] These infections can be classified as early (<1 month), intermediate (1-6 months), or late (>6 months) infections. Generally, early infection etiologies most frequently include bacteria or Candida[17], while intermediate infections are typified by the viral infections such as cytomegalovirus, Epstein-Barr virus, varicella zoster virus, herpes B virus, human herpesvirus-6, and simian immunodeficiency virus.[10] It is probably more accurate to consider these as re-emergent latent pre-transplantation viral infections rather than de novo post-operative infections.



Lung, rhesus macaque. There are multifocal areas of septal necrosis and thrombosis (arrows) throughout the section. (HE, 220X).

In humans, CMV is most commonly associated with HIV and transplant-associated immunosuppression.[8,19,20] CMV-related diseases in these cases include chorioretinitis, gastrointestinal diseases (colitis, esophagitis, gastritis, hepatitis), pneumonia, encephalitis and myelitis, and adrenal adenitis. Lesions associated with cytomegaloviral infection in nonhuman primates result from disseminated infections, which are almost always linked to immunosuppression. Diseases resulting from disseminated infection include: necrotizing enteritis, encephalitis, lymphadenitis, and/or pneumonitis/interstitial pneumonia.[1] Typically, alveolar septa are lined by hypertrophied type 2 pneumocytes and contain cytomegaly with large intranuclear Cowdry type-A inclusion bodies within the septa and lining. Alveolar spaces contain fibrin, alveolar macrophages, and neutrophils. Similar lesions can also be found in spleen, liver, kidney, and testis. The similarities between humans and nonhuman primates regarding pathogenesis and gross and histologic lesions makes nonhuman primates an ideal model for studying CMV in immunosuppressed patients.

Within the present case, only pulmonary lesions (and resultant hydrothorax) were observed, with classical cytomegaloviral inclusions observed predominately within vascular endothelial cells, and alveolar septa and macrophages much less frequently observed. Endotheliitis in the lungs and other organs from cytomegalovirus has also been described in immunosuppressed humans.[9,21] Vasculitis is a less common pathologic finding than alveolitis, but can cause perivascular and alveolar edema and hemorrhage, as well as pleural effusion.[11,18] In rhesus macaques, CMV has also been reported to cause hypophysitis[5] and facial neuritis[3] with co-infection with SIV, and mesenchymoproliferative enteropathy with co-infection with SIV and simian polyomavirus.[14]

Contributing Institution:

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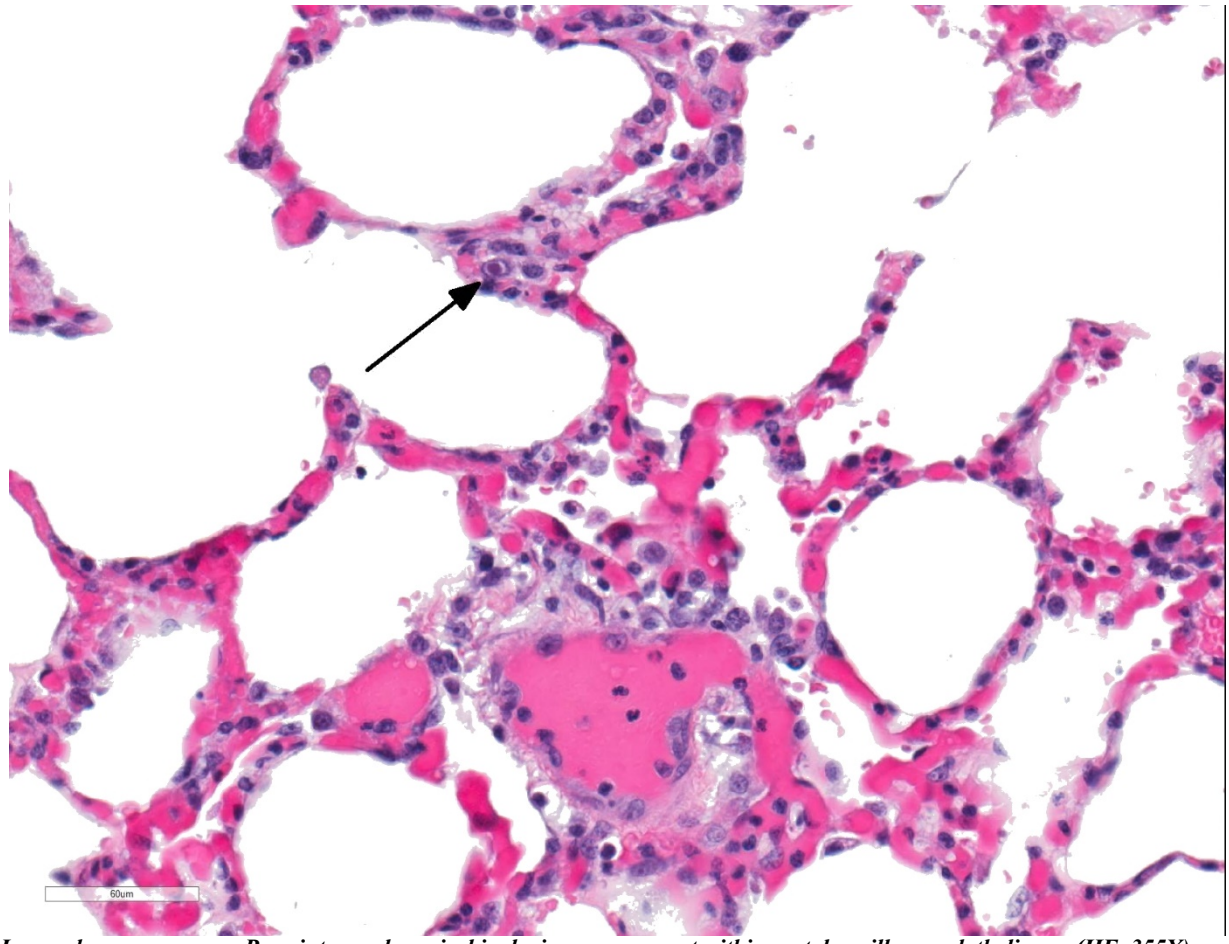
http://www.yerkes.emory.edu/research/divisions/pathology/index.html

JPC Diagnosis: Lung: Pneumonia, interstitial, necrotizing and neutrophilic, multifocal to coalescing, moderate, with necrotizing vasculitis and numerous endothelial and pneumocyte karyomegalic viral inclusions.

JPC Comment: The contributor has provided an excellent and concise review of cytomegalovirus, and their importance in both primate and transplant research.

Present in most if not all mammalian species, cytomegaloviruses (CMV) are slow-growing viruses that result in a life-long latent infection, with recrudescence and clinical disease only in times of severe immunosuppression. In order to do this, cytomegaloviruses have developed unique methods of preventing the host from clearing the infection, which are only now beginning to come to light.



Lung, rhesus macaque. Rare intranuclear viral inclusions are present within septal capillary endothelium. (HE, 355X).

As shown in the mouse model (murine cytomegalovirus is a well-researched agent due to its similarity to human CMV), toll-like receptors and cellular stress responses will activate intrinsic methods of cell death following CMV infection. In order to combat this, CMV has developed ways to counteract a number of key factors which inhibit cellular death, i.e., "death inhibitors".[6]

One method is a viral protein which inhibits mitochondrial outer membrane permeabilization, a key step in apoptosis, by producing vMIA (viral mitochondria-localized inhibitor of apoptosis), which inhibits the activator Bak and sequesters Bax at the mitochondrial membrane.[6]

CMV also produces a viral protein, UL38, which results in accumulation of the activating transcription factor 4 (ATF4) and suppression of JNK activity. ATF4 helps to resolve cell stress by inducing the production of proteins that facilitate protein folding within the ER, and the inactivation of JNK inhibits activation of Bim and Bcl-2, further stabilizing the mitochondrial membrane.[6]

CMP infection also modifies so-called "death receptors" at the cell surface, such as the TNF-related apoptosis-inducing ligand (TRAIL). An open reading frame on M166, a CMV viral-encoded protein inhibits the expression of this receptor at the cell surface. Other viral proteins inhibit Fas-induced cell death by binding to pro-caspase

8 and inhibiting its activation.[6] Several proteins also inhibit activators of null killer cells at the level of the NKG2D cell receptor (CD226).[7]

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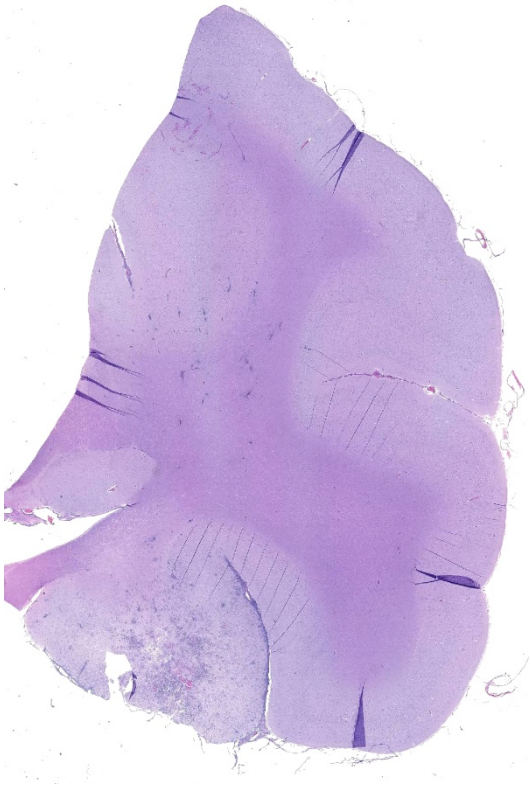
CASE III: 61726-1 (DVD) (JPC 4117676)**.**

Signalment: 2-year-old, female Angolan Colobus monkey (Colobus angolensis palliatus)

History: This monkey was found down and unresponsive at morning check and transported to the hospital immediately. She



Cerebrum, colobus monkey. A large area of necrosis involving the grey matter is present at lower left. There are multifocal areas of hypercellularity outlining vessels within the white matter of the corona radiata and adjacent grey matter. (HE, 5X)

had no significant history prior to this event, and keepers reported no recent cause for concern. On physical examination she was obtunded and ataxic, with decreased lung sounds on the left and mild crackles on the right. A small abrasion was present on the left ischial pad. On a CT scan, there was a severe, diffuse, nodular/bronchiolar pattern throughout the lungs with a left-sided alveolar pattern. No apparent soft tissue asymmetry was noted. The animal failed to respond to supportive care and was humanely euthanized later the same day due to poor prognosis.

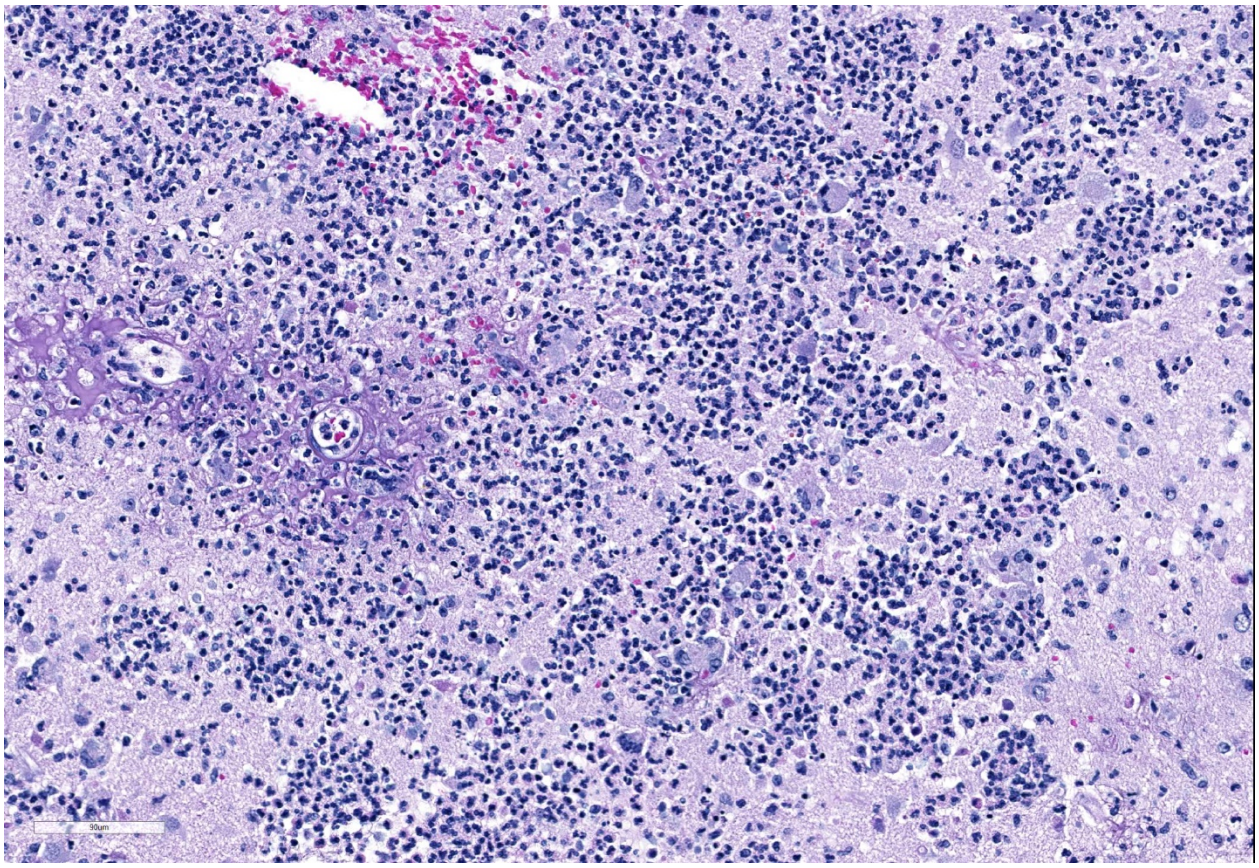
Gross Pathology: Postmortem examination revealed evidence of interstitial pneumonia, nephritis characterized by coalescing tan, red-rimmed nodules, and a prominent focus of hemorrhage in the rostral left cerebrum.

Laboratory results:

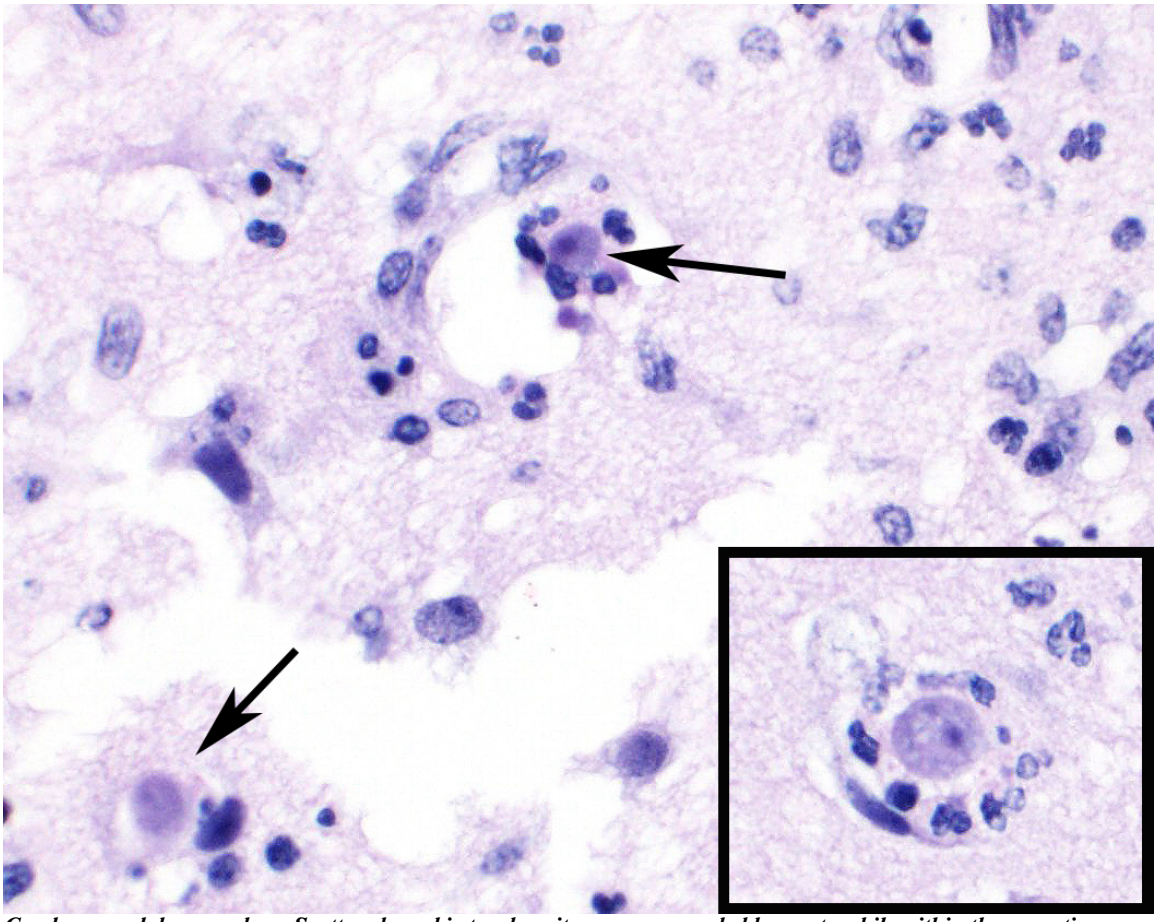
Clinical pathology:

- A CBC showed a mild non-regenerative anemia (HCT 30), thrombocytopenia (158,000/ul), and increased fibrinogen (400 mg/dl). A serum chemistry was unremarkable.

Microscopic Description: Cerebrum: Approximately 15-20% of the parenchyma, of which the majority is gray matter, is affected by multifocal to coalescing



Cerebrum, colobus monkey. The affected area is infiltrated by innumerable viable and degenerate neutrophils and few macrophages/Gitter cells and foreign body-type macrophages. Vessels are necrotic with exudation of fibrin into the surrounding parenchyma. (HE, 242X)



Cerebrum, colobus monkey. Scattered amebic trophozoites are surrounded by neutrophils within the necrotic area. (HE, 242X)

rarefaction, necrosis, and hemorrhage, and is infiltrated by large numbers of neutrophils with fewer histocytes and lymphocytes. Vessel walls in this region are frequently diffusely hyalinized, disrupted and expanded by eosinophilic fibrillar material and karyorrhectic debris (fibrinoid necrosis), and infiltrated by neutrophils (vasculitis). Moderate numbers of amoebic trophozoites measuring from 15-30 micrometers in diameter with a 4-6 micrometer karyosome (nucleus), up to three endosomes (nucleoli), and amphophilic, lacy to vacuolated cytoplasm are scattered throughout the inflamed region. There are increased numbers of glial cells (gliosis), and astrocytes have large nuclei with open chromatin (reactive astrocytosis). The meninges are expanded by large numbers of lymphocytes, plasma cells, and histiocytes with fewer neutrophils. Vessels in surrounding parenchyma are surrounded by large numbers of lymphocytes and plasma cells, which multifocally infiltrate vessel walls.

Immunohistochemistry:

Balamuthia immunohistochemistry: positive

Acanthamoeba immunohistochemistry: negative

Naegleria immunohistochemistry: negative

Contributor's Morphologic Diagnosis:

Brain, cerebrum: Severe, marked, regionally extensive, necrotizing, pyogranulomatous, meningoencephalitis with cavitation, fibrinoid vascular necrosis, vasculitis, and amoebae.

Contributor's Comment: Infections with free-living amoeba are an emerging disease in both human and animal populations. The major differentials for free-living amoeba infecting animal and human tissues include *Balamuthia* sp, *Acanthamoeba* sp, and *Naegleria fowleri*. These three organisms can be difficult to distinguish from each other based on light microscopy alone except for a few distinguishing features. The distinguishing feature of *Naegleria fowleri*, is that it does not form cysts in the brain, whereas *Balamuthia* sp. and *Acanthamoeba* sp. do.[8]

Acanthamoeba and *Balamuthia* are very similar morphologically and can be difficult to diagnose without additional diagnostics such as immunohistochemistry or PCR. One feature that may assist in distinguishing between the two is that *Balamuthia* can have multiple nucleoli within its trophozoite stage, whereas *Acanthamoeba* only has one nucleolus. Also, *Balamuthi*a trophozoites are slightly larger and tend to be more pleomorphic than *Acanthamoeba* trophozoites.[5] The size of the amoeba (trophozoites 15-30 micrometers in greatest dimension) along with the occasional presence of multiple endosomes or nucleoli in this case is consistent with *Balamuthia mandrillaris*, which was subsequently confirmed by immunohistochemistry.

Balamuthia mandrillaris was first discovered in a 3-year old female mandrill baboon

(*Mandrillus sphinx*) from the San Diego Zoo Safari Park that died of amoebic encephalitis in 1986. The species name *mandrillaris* comes from this index case.[7] Since the first case, additional sporadic cases have been identified at the San Diego Zoo and San Diego Zoo Safari Park over the years.[5] In addition, the agent was subsequently found to be an important human pathogen, explaining a number of historical human cases of amoebic encephalitis that failed to stain with antibodies against *Acanthamoeba* and *Naegleria.*[1]

Balamuthia mandrillaris is a soil-dwelling organism. The pathogenesis of the infection remains uncertain, but open skin lesions are thought to be a risk factor in human cases.[6] The animal in this case did have an abrasion on an ischial pad, which could have been a portal of entry. Exposure to blowing dust, mud puddles, or other soil contaminated water are thought to be additional potential risk factors.[4] These infections are not transmissible and are not zoonotic. Individuals infected with free-living amoeba tend to be immunocompromised in some way, with a number of human patients being infected with AIDS[6]; however, immunocompromise is not required for infection. Morbidity is overall low, but mortality is relatively high with human and animal infections.[4]

Common organ systems infected in humans include skin and the central nervous system. Lesions are typically granulomatous and necrotizing. Unlike other free-living

amoeba, *Balamuthia mandrillaris* can cause disseminated chronic infections that are indistinguishable from metastatic neoplasia.[1] Overall, free-living amoeba infections are important to have as a differential for various presentations in both animal and human patients.

Contributing Institution:

San Diego Zoo Global Disease Investigations
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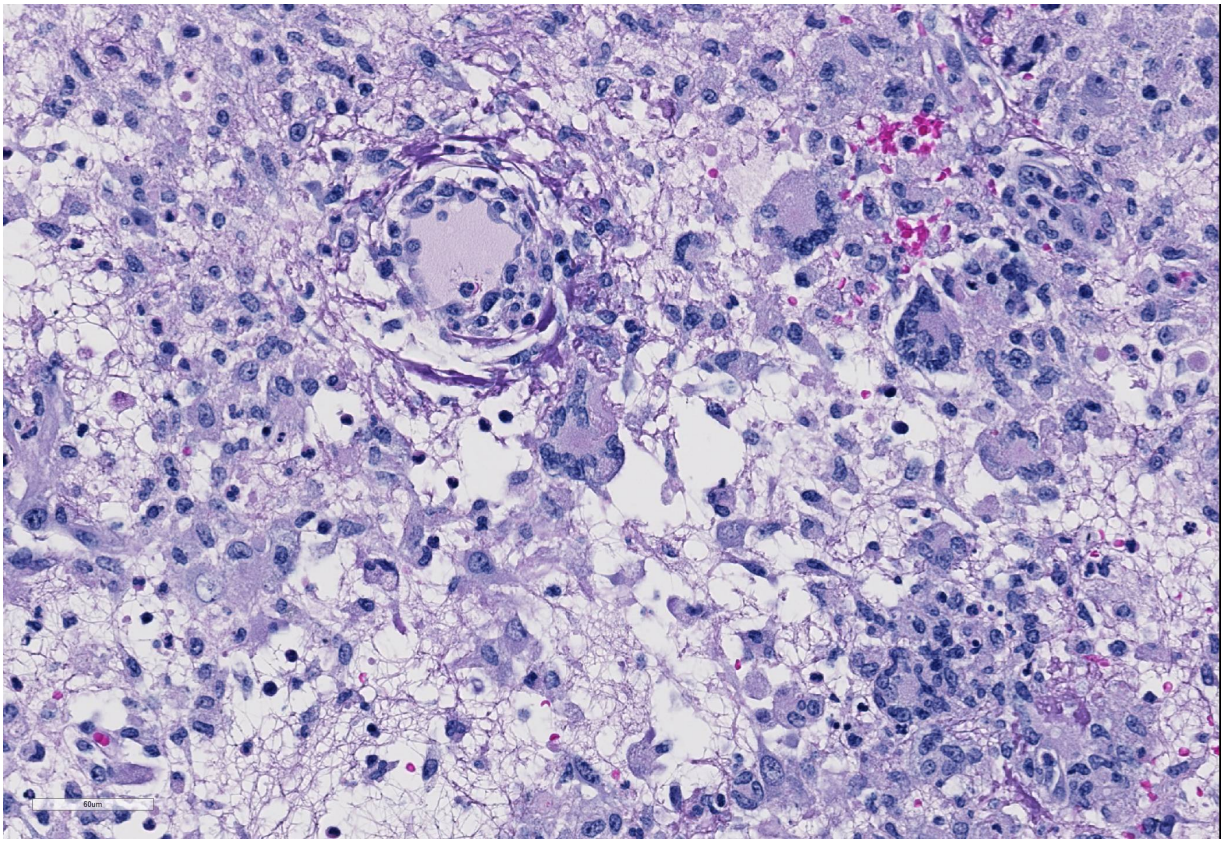
San Diego, CA 92112-0551
https://institute.sandiegozoo.org/disease-investigations

JPC Diagnosis: Cerebrum: Meningoencephalitis, necrotizing and pyopgranulomatous, focally extensive, severe with vasculitis, thrombosis, gliosis, and numerous amebae.

JPC Comment: Free-living amebae are ubiquitous protozoans in the environment, of which four generally are considered pathogenic for humans and animals: *Acanthamoeba*, *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia pedata.*[1,3] As a group, they may act as vectors for a wide range of pathogenic bacilli, as well as hosts for a range of viruses, including coxsackieviruses and adenoviridae pathogenic for humans. Other viruses, the so-called giant viruses, may act as



Cerebrum, colobus monkey. In more longstanding areas of infection, liquefaction predominates with large numbers of uni- and multinucleated Gitter cells. (HE, 348X)

endocytobionts, including representatives of the *Mimi*-, *Moumo*- and *Megaviridae*, as well as *Pandoviridae*. Human infections with free-living amebae, while uncommon, are especially problematic due to their high mortality, non-specific symptoms, and lack of effective treatment.

Balamuthia mandrillaris is also a cause of granulomatous amebic encephalitis which usually results from hematogenous spread from soil-contaminated wounds and which ranges in duration between *Acanthamoeba* and *Naegleria* (discussed below). A recent review of human cases from the CDC's free-living ameba registry identified 109 cases in the US within the last forty years[3] with 99% resulting in encephalitis; 6% had skin lesions as well. 68% of cases were male, with people of Hispanic ethnicity most frequently affected, and California, Texas, and Arizona had the most cases. Immunosuppressed patients accounted for less than 40% of this study. Due to the non-specific clinical signs and laboratory diagnostics, only 25% of patients received an antemortem diagnosis of *Balamuthia* infection.[3]

In humans, *Balamuthia* is thought to enter by inhalation or cutaneous wounds; hematogenous spread is supported by the tendency for trophozoites to cluster around vessels, as well as its propensity to infect multiple organs. In 2009, organ transplantation was identified as another method of transmission with kidney recipients of a donor that died of *Balamuthia* encephalitis.[3]

Acanthamoeba appear to be most often associated with disease in humans and animals, with 18 distinct genotypes based on nuclear small-subunit ribosomal DNA rather than morphology. The most common condition associated with infection in humans is a chronic keratitis, seen in immunocompetent patients associated with improper handling of contact lenses, exposure to contaminated water, or trauma. Risk factors of contact lens users include the use of all-in-one solutions, showering while wearing contact lenses, and poor contact lens hygiene.[4] Other species of free-living amoeba which may have been identified in cases of keratitis include *Hartmanella, Vahlkamphiae*, and *Allovahlkampfiae spelae.*[4]

Granulomatous amebic encephalitis is a well-documented syndrome in humans resulting from hematogenous spread, often from the lower respiratory tract or skin lesions. It shows a chronic fatal progression with luckily only 150 documented cases worldwide.[4] Due to its hematogenous origins, areas of granulomatous inflammation are seen throughout all parts of the brain. Cutaneous acanthamoebiasis is also an uncommon opportunistic condition primarily seen in immunosuppressed patients, resulting in erythematous sores and skin ulcers.[4]

Primary amebic meningoencephalitis (PAM) is another rare fatal disease in humans caused by *Naegleria fowleri.* This condition generally occurs in healthy children and adults swimming or bathing in in warm freshwater ponds. Infective amebae migrate along olfactory nerves from the nose to the

brain; fatal infection proceeds rapidly and is almost always fatal. Due to this unique entry portal, areas of lytic necrosis are clustered at the base of the brain, hypothalamus, pons, and occasionally seen in posterior areas such as the medulla oblongata.[4]

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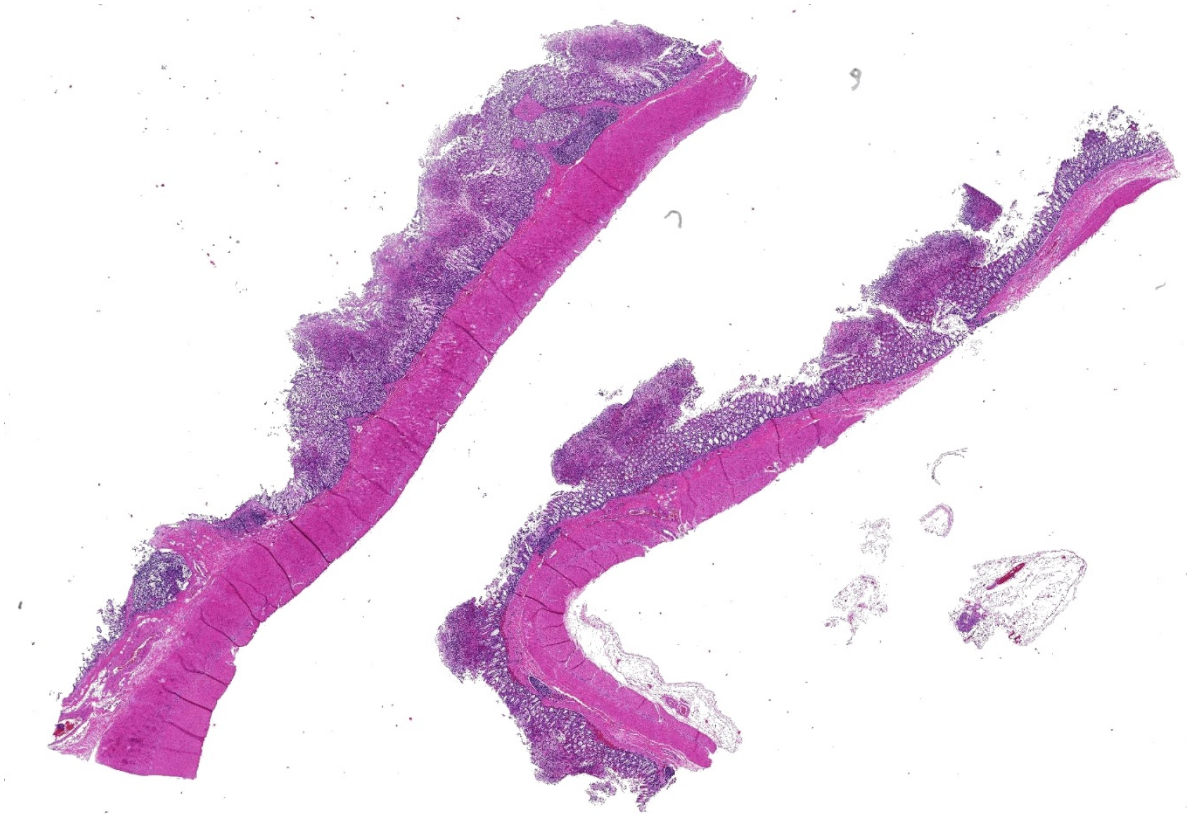
CASE IV: WSC 1920 Conf 22 Case 4 DVD (JPC 4100234).

Signalment: 26-year-old, male intact Geoffroy's spider monkey (*Ateles geoffroyi)*

History: The previous year, this animal had episodes of depression and diarrhea and was diagnosed with *Campylobacter hyointestinalis*. At that time, treatment led to complete resolution of signs. The following year, the monkey presented with lethargy and loose stool. Weight loss of 2 kg had occurred over the previous two months and symptomatic treatment including fluids and antibiotics was provided. The animal died the following day.

Gross Pathology: On necropsy, the markedly dilated large intestine contained a large amount of homogenous, brownish, turbid, thick fluid. The most aborad ileal mucosa, the cecal mucosa, and large



Intestine, spider monkey. Multifocally, the mucosa is effaced by foci of eosinophilic cellular debris that occasionally extends superficially and laterally from the mucosa surface. (HE, 5X)

portions of the colonic mucosa were covered by pale brown membranous material that was easily removable. Underneath this material, the mucosa was reddened but appeared otherwise intact. While the cecal mucosa was almost entirely coated by the pseudomembranous material, particularly in the colon, the pseudomembraneous material was distributed multifocally in numerous geographical to round, up to 3 to 4mm in diameter convex deposits that were roughly evenly spaced.

Laboratory results:

Bacteriology – aerobic and anaerobic cultures were negative. A culture for *Clostridium difficile* specifically was positive. There was no evidence of a parasitic infection based on the negative result of fecal flotation. Viral particles were not detected in the colonic content at electron microscopy.

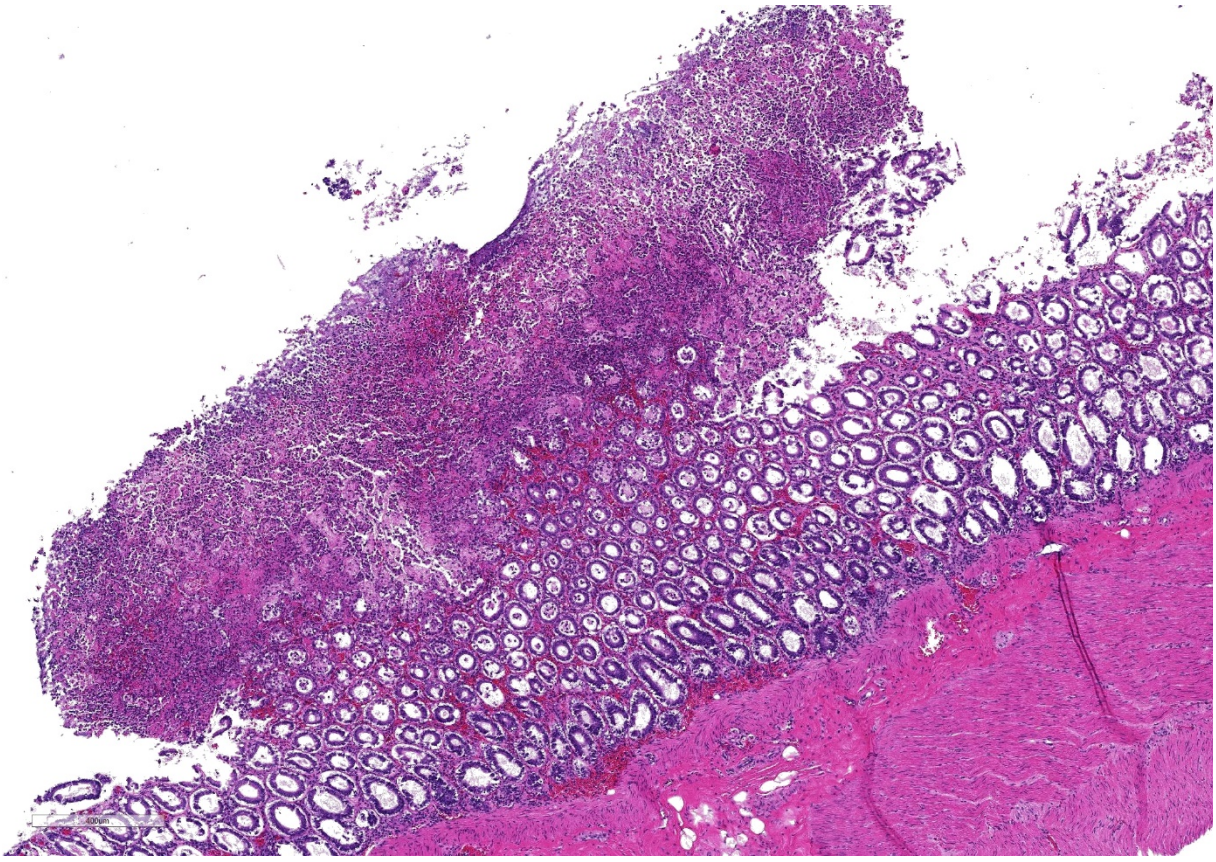
Microscopic Description: Two to three full-thickness sections of similarly affected cecum and colon are evaluated. Multifocally, there are 'volcano lesions' composed of aggregations of sloughed epithelial cells, numerous degenerate and non-degenerate neutrophils, few erythrocytes, and fibrin within and extending from the underlying eroded epithelium and lamina propria into the lumen. Along the luminal aspect of these aggregates, frequent individual and clustered rod-shaped, straight pale basophilic bacteria up to 10 um long, occasionally with a visible

clearing (oval spores), are admixed with cocci-shaped and shorter rod-shaped bacteria. The underlying epithelium is intact within some of these areas and partially to completely necrotic in others, with variable extension of inflammatory infiltrates into the mucosa. The superficial mucosa subjacent to some of these lesions is necrotic, with sparing of the remainder of the crypts. The lamina propria is moderately expanded by plasma cells, lymphocytes, and degenerate leukocytes. Necrosis extends full thickness overlying some of the underlying lymphoid follicles (Peyer's patches/gastrointestinal-associated lymphoid tissue) within the sections of ileum and cecum. Small aggregates of fibrin are present within the lumina of a small to moderate number of the capillaries of the lamina propria and submucosa. The submucosa is multifocally expanded by small numbers of lymphocytes, plasma cells, and lower numbers of eosinophils, with mild to moderate increased submucosal clear space within some sections (edema). There is mild to moderate submucosal congestion.

Contributor's Morphologic Diagnosis:

Cecum and colon, typhlocolitis, erosive and fibrinonecrotizing (pseudomembranous), multifocal, marked, acute

Contributor's Comment: *Clostridium difficile* is a toxin-producing, gram-positive, spore-forming anaerobe best known for its role in causing pseudomembranous colitis in



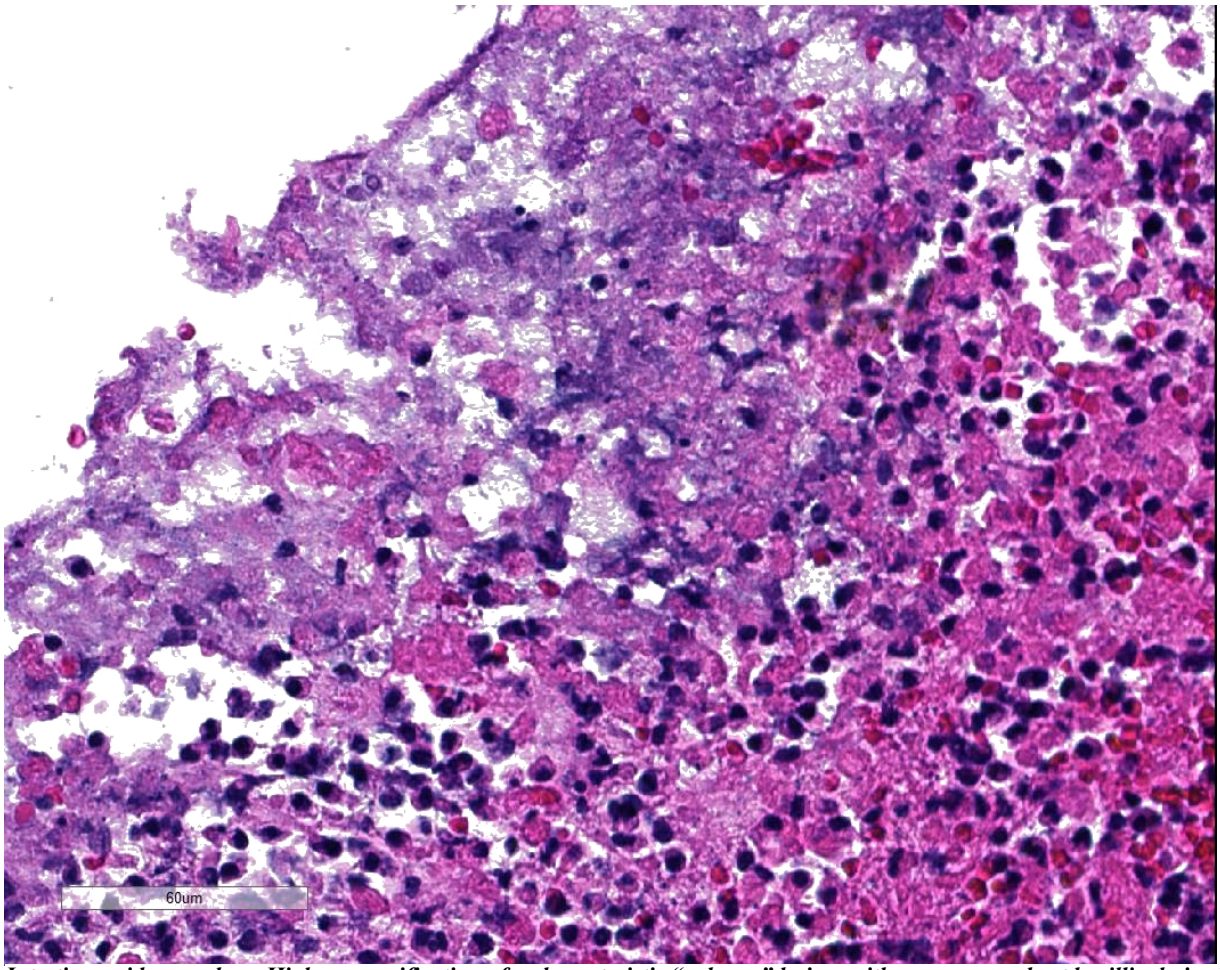
Intestine, spider monkey. Higher magnification of a characteristic "volcano" lesion, with abundant necrotic cellular debri arising over the mucosa. (HE, 20X)

humans (often referred to as *Clostridium difficile*-associated disease or CDAD), often associated with antibiotic administration.[5] As use of antibiotics has increased, so has reported cases of pseudomembranous colitis, with *C. difficile* acting as a source of a potent enterotoxin. To the author's knowledge, *Clostridium difficile*-related pseudomembranous colitis with characteristic 'volcano' lesions has not been described in New World monkeys, with a single reported case in a marmoset within the 2016 Wednesday Slide Conference that displayed more severe and diffuse histologic changes and a case report of several cotton-top tamarins that died suddenly following antibiotic therapy for *Campylobacter spp.* associated diarrhea, with identification of *Clostridium difficile* toxin within the feces in all and pseudomembranous colitis in two of the five described cases.[8]

In humans, several papers have described various morphologic stages of lesion in cases of pseudomembranous colitis, beginning with a focal epithelial necrosis with exudation of fibrin and neutrophils, followed by presence of a marked exudate extending through the area of mucosal ulceration and forming the classic "volcano" lesion, ultimately leading to a more diffuse and severe mucosal ulceration and necrosis with presence of a pseudomembrane.[5,7,10] In this case, the volcano-like lesions were a prominent feature. Similar 'volcano lesions' have been described within piglets diagnosed with *Clostridium difficile*-associated typhlocolitis and within mice and hamsters and are reported rarely in horses.[2,3,6]

While CDAD is considered well-described in humans and has been characterized in several animal species, the challenges of definitive diagnosis are ongoing. Cultures of *Clostridium difficile* are capable of isolating the bacteria, but interpretation of results is not clear-cut, given the isolation of *C. difficile* in low numbers from asymptomatic animals that have not been treated with antibiotics and in higher numbers from asymptomatic animals following antibiotic treatment.[2,13] For this reason, toxinotyping of cultured isolates is necessary to rule out presence of a non-toxigenic strain of *C. difficile*, though this is more readily available in human medicine than in veterinary medicine. Toxinotyping is a PCR-restriction fragment length polymorphism-based method that classifies strains of *C. difficile* based on variations in the pathogenicity locus (PaLoc), which is the region that codes for toxin A and toxin B, and groups strains by those with identical changes within the PaLoc region. Many isolates of *C. difficile* have been identified with varying toxigenic properties, with 34 toxinotypes reported based on sequence variations in the A and B toxin genes. PCR ribotyping allows further characterization of strains of *C. difficile* and their relatedness, which has been of particular interest due to geographical variation in the prevalence of various ribotypes.[14]

The primary virulence factors of *C. difficile* are two major exotoxins, toxin A and toxin B. Toxin A and Toxin B are both enterotoxins, while Toxin B is also a cytotoxin. Alone or together, these toxins have the ability to glycosylate and inactivate Ras GTPases, disabling key cell signaling



Intestine, spider monkey. Higher magnification of a characteristic "volcano" lesion, with numerous robust bacilli admixed with degenerating neutrophils, hemorrhage, and cellular debris. (HE, 400X)

pathways, and glycosylate Rho and interfere with its regulation of cytoskeletal actin.[14] Fecal ELISA is commonly used to identify one or both of these toxins, and was utilized in this case to confirm the presence of *Clostridium difficile* A and B toxins in three of four other similarly affected monkeys within the same colony, though samples were not available for submission from this particular individual at the time of diagnosis.

Almost any antibiotic can cause disruption of the intestinal microbiota and subsequent *Clostridium difficile* infection, with clindamycin frequently implicated in human cases, along with other antibiotics such as cephalosporins and broad-spectrum penicillins that are widely prescribed.[5] Resolution of clinical signs is often successful with vancomycin or metronidazole treatment.[13] *C. difficile* infection has been more widely reported in the equine and implicated in gastrointestinal disease in the dog and cat, though there is controversy as to the importance of antibiotic exposure as a risk factor in development of CDAD in these species.[2,12] Diet change and environmental stressors have also been implicated in disruption of the gastrointestinal flora and subsequent

colonization and development of CDAD in several species.[2,6]

Contributing Institution:

University of Minnesota Department of Veterinary Population Medicine/Minnesota Veterinary Diagnostic Laboratory - https://www.vetmed.umn.edu/departments/veterinary-population-medicine ; https://www.vdl.umn.edu/

JPC Diagnosis: Cecum, colon (per contributor): Typhlocolitis, necrotizing, multifocal to coalescing, marked, with numerous extracellular bacilli.

JPC Comment: *C. difficile* has been the cause of disease in a wide variety of mammalian species. First identified from feces of clinically healthy human babies in the 1930s, the organism was originally named *Bacillus difficilis* because of the difficulties encountered in cultivating it. In humans, most infected people will remain asymptomatic, with the remainder developing variable GI signs ranging from watery diarrhea to pseudomembranous colitis[1], particularly if they have been recently hospitalized or the recipient of antibiotics.

In humans, *C. difficile* associated disease (CDAD) was always assumed to affect individuals of any age except during the neonatal period, as it was thought that this specific group may lack specific *C. difficile* toxin receptors. Although between 25 and 70% of human neonates are colonized with *C. difficile*, these microorganisms have been largely considered part of the commensal microbiota. Recently, however, two 9- and 18- month-old children were diagnosed with CDAD, providing evidence that *C. difficile* is a potential cause of bloody diarrhea in neonates and young infants. In most animal species, CDAD is not age-dependent. The exception to this are pigs, which are almost exclusively affected during the neonatal period, up to approximately one week of age.[11]

The gut microbiota is the primary protection against *C. difficile* overgrowth and overt disease. In addition to antibiotic administration, alteration in bile acids (which promote the germination of *C. difficile)* has been noted in affected human patients, adding another potential factor in CDAD.[1] An emerging treatment for recurrent *C. difficile* infection is fecal microbiota transplants, which showed significantly higher rates of resolution of recurrent CDAD than those with conventional antibiotic treatment. In the Netherlands, one large medical center has set up the "Netherlands Donor Feces Bank" and a panel of internal medicine and infectious disease specialists review each case to ensure it fits a rigid set of inclusion criteria, accepting 80% of referrals and posting a 90% rate of success.[1]

In clinical disease, the bacillus is not infective. Mucosal necrosis and loss of barrier integrity is the result of liberation of a number of enzymes, including collagenase, hyaluronidase, and chondroitin-sulfatase, as well as the previously mentioned toxins, which act on the epithelial cell cytoskeleton, leading to enterocyte disassociation, fluid loss, and local inflammation.[1]

C. difficile-associated disease (CDAD) affects a wide range of other mammals – while always resulting in enterocolitis, its manifestation varies widely with the affected species. Table 1 summarizes the severity of disease in various species by enteron segment.

Table 1. Summary of lesion distribution by species.[3]

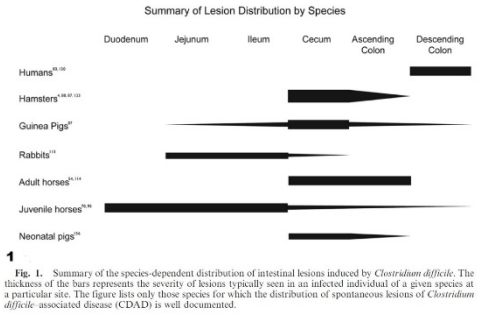


Fig. 1. Summary of the species-dependent distribution of intestinal lesions induced by *Clostridium difficile*. The thickness of the bars represents the severity of lesions typically seen in an infected individual of a given species at a particular site. The figure lists only those species for which the distribution of spontaneous lesions of *Clostridium difficile*–associated disease (CDAD) is well documented.

In rodents, CDAD is primarily cecal, resulting in ulcerative and rarely proliferative typhlitis and death. In pigs, the disease results in ulcerative typhitis or colitis with development of "volcano ulcers". An additional gross finding of mesocolonic edema[4] (as well as diarrhea) makes CDAD a differential diagnosis for edema disease in swine.[2] The difference is that *C. difficile* infections occurs in young piglets (1-7 days of age), while edema disease is a disease of weanling age pigs. It has been suggested as a pathogen for enteritis in young calves[4] as well as an agent that can cause necrotic enteritis in poultry.[10] In some Latin American countries, a linkage between high incidence in poultry and high prevalence of CDAD in humans has been identified.[4] In rabbits, the lesion is primarily seen in the small intestine and concentrated in the ileum, often following antibiotic administration. It has also been reported sporadically in dogs, cats, ostriches, prairie dogs, and experimentally in non-human primates.[11]

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