Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2016-2017

C o n f e r e n c e 3

7 September 2016

CASE I: CHIN1 (JPC 4084953).

Signalment: One-year-old female chinchilla (*Chinchilla lanigera*).

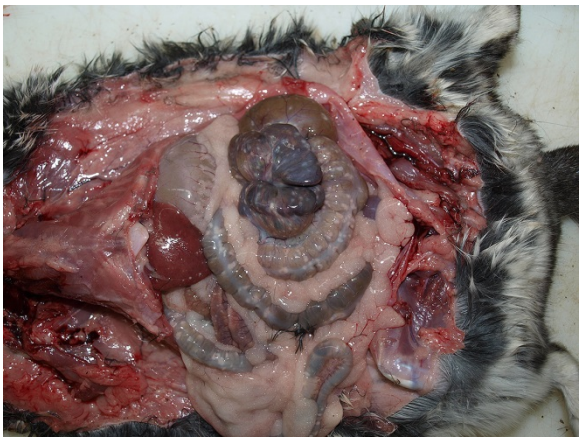
History: The owner had three chinchillas. Through the summertime, they were held in a fenced part of the garden. Animals were fed with guinea pig chow, apples, and raisins. Four days before death, they refused food, and one animal circled. All animals died and one was submitted for necropsy.

Gross Pathology: The liver was slightly enlarged with numerous pinpoint necrotic foci. Multifocal randomly distributed white foci were also visible throughout the small and large intestine, and few foci were in the myocardium.

Laboratory results: *Listeria monocytogenes* was isolated on bacterial culture of the liver.
Tissue gram stain: in necrotic foci, there are numerous intra- and extracellular road-shaped bacteria consistent with *Listeria monocytogenes*.

Histopathologic Description: Liver: There are multifocal, randomly distributed foci measuring 100-200 µm characterized by hepatocyte loss which are replaced with necrotic cellular debris and moderate numbers of degenerate neutrophils (lytic necrosis) and few macrophages. Often, necrotic foci contain variable numbers of intracellular and extracellular rod-shaped bacteria. Multifocally, randomly distributed smaller foci of hepatocytes are characterized by swollen, hypereosinophilic cytoplasm and loss of nuclei (coagulative necrosis). Throughout the liver are moderate numbers of hepatocytes that contain distinct cytoplasmic vacuoles consistent with lipid. Multifocally, mainly in periportal areas, there are scant infiltrates of lymphocytes and plasma cells.

Contributor's Morphologic Diagnosis: Liver: Moderate to severe, multifocal and random necrotizing and suppurative hepatitis with rod-shaped bacteria consistent with *Listeria monocytogenes*.

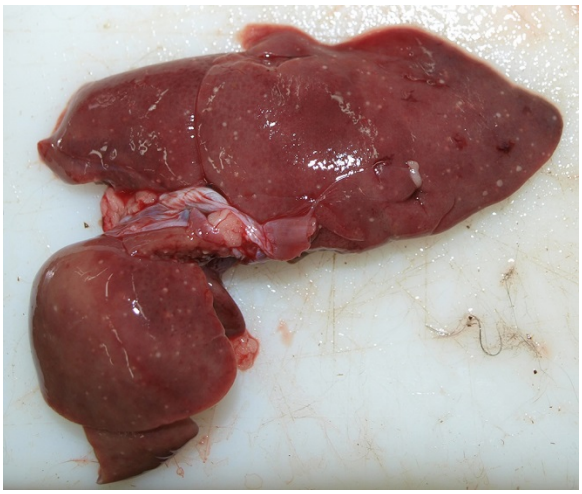


Abdominal viscera in situ, chinchilla: Numerous pinpoint foci are present within the liver and throughout the small and large intestine. (Photo courtesy of: Department of Veterinary Pathology, Faculty of Veterinary Medicine University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia. http://www.vef.unizg.hr/)

Contributor's Comment: Listeriosis is caused by *Listeria monocytogenes*, a gram-positive, facultatively anaerobic bacillus that is ubiquitous in the environment. The organism is commonly isolated from tissues of normal animals, including tonsils, other gut-associated lymphoid tissue, and feces of ruminants. The bacterium has also been isolated from soil, animal feed, water, and improperly stored silage.[1, 9] *Listeria monocytogenes* has more than 11 serotypes; almost all animal infections are caused by serotypes 1/2a, 1/2b, and 4b.[1]

Listeria monocytogenes is a facultative intracellular pathogen that invades host macrophages, neutrophils, and epithelial cells. Important virulence factors include the surface protein internalin, which interacts with host cell E-cadherin on the host cells, allowing the bacterium to cross the intestine, placenta, and blood-brain barrier. Once inside the cell, the organism also utilizes cholesterol-binding hemolysin to lyse phagolysosomes and escape into the cytoplasm. The organism proliferates in the host cell cytoplasm and migrates against the cell membrane to form protrusions that can then be taken up by adjacent cells.[1]

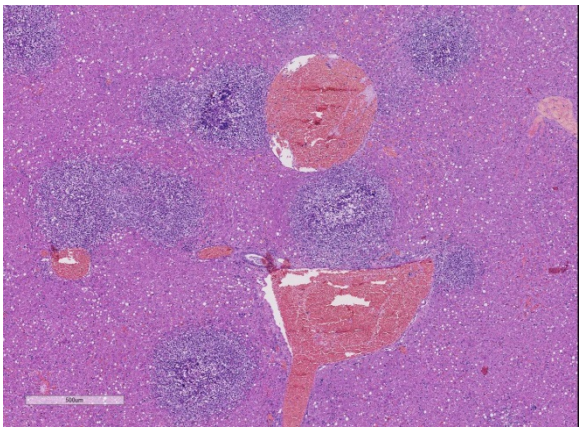
Listeria monocytogenes behaves as three separate rarely overlapping diseases or syndromes: infection of the gravid uterus with abortion; septicemia with miliary visceral abscesses or necrosis; and encephalitis.[1] Septicemic (systemic) listeriosis occurs in aborted fetuses and neonatal lambs, calves, foals up to one week of age, and young rabbits; it is characterized by multisystemic bacterial colonization and multifocal multisystemic areas of coagulative necrosis or microabscesses formation.[1, 7] Necrotic foci are numerous in the liver, but much less numerous in the heart and other viscera. Neonates generally become infected in utero.[1]



Liver, chinchilla. Closer view of the liver, with multiple foci of pinpoint necrosis scattered throughout all lobes. (Photo courtesy of: Department of Veterinary Pathology, Faculty of Veterinary Medicine University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia. http://www.vef.unizg.hr/)

Chinchillas are particularly susceptible to infection with *Listeria monocytogenes* and most reports mention massive outbreaks involving a significant number of animals on chinchilla farms.[3, 8] A single case report of listeriosis in a chinchilla caused by *Listeria*

ivanovii has been described, and lesions were also characterized with necrotic and suppurative hepatitis.[2] Currently, chinchillas are becoming more common as pets, and less are kept for fur production. In the presented case, the source of infection remains unknown, but it may be speculated that infection occurred due to contamination of water or food during the time when animals were kept in the garden.

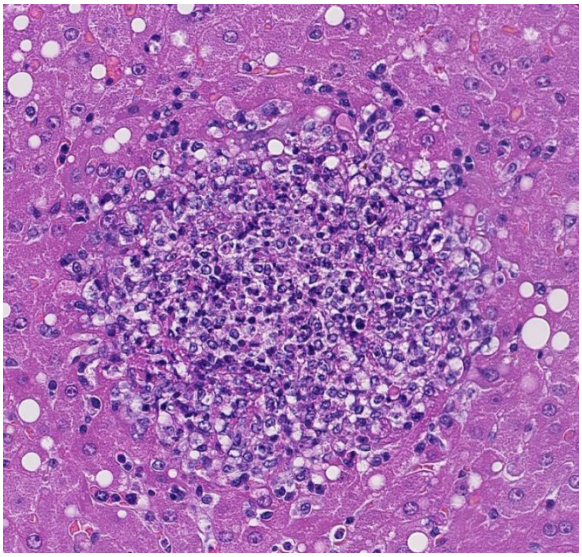


Liver, chinchilla: Areas of lytic necrosis are scattered randomly throughout the hepatic parenchyma. (HE, 40X)

JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal, random, marked, with intrahistiocytic and extracellular bacilli, chinchilla (*Chinchilla lanigera*).

Conference Comment: The contributor provides a thorough overview of the clinical manifestations, epidemiology, and pathogenesis of *Listeria monocytogenes* in the highly susceptible chinchilla. Most cases of listeriosis in humans and animals are secondary to ingestion of contaminated food and water, and the disease is particularly common in ruminants fed improperly fermented and stored silage.[1,2,6,8] Chinchillas, like rabbits and guinea pigs, are monogastric hind-gut fermenters and are more susceptible to the septicemic form of this disease rather than the encephalitic form, the typical manifestation in adult ruminants.[5,6]

After ingestion of the bacteria and translocation from the intestinal tract, the main target organ is the liver.[1,4,6] The organism has a tropism for hepatocytes and has the ability to penetrate the host cell using the surface protein internalin, mentioned by the contributor.[1] The bacterium then replicates within the host cell cytoplasm. *Listeria monocytogenes* also has the ability to co-opt the host cell actin filaments using the bacterial actin assembly-inducing protein (ActA) to migrate to the host cell membrane and induce pseudopod-like protrusions that can be transferred to another host cell.[4] Subsequent recruitment of neutrophils leads to lysis of hepatocytes, release of the organism, and ensuing

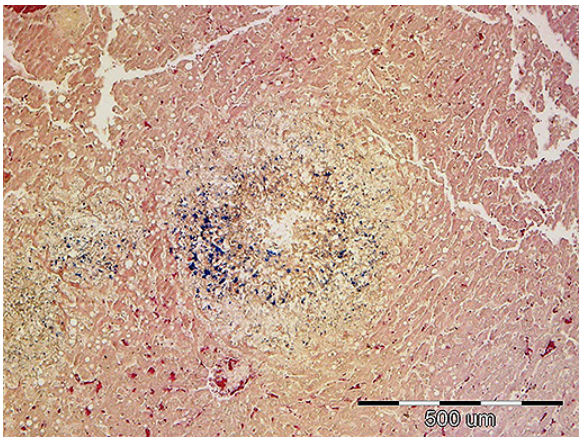


Liver, chinchilla: Necrotic foci are composed of moderate numbers of viable and degenerate neutrophils admixed with moderate numbers of infiltrating macrophages and cellular debris. (HE, 175X)

bacteremia.[6] Dissemination of the organism to multiple tissues such as the heart, brain, spleen, lymph nodes, and intestinal tract causes the classic lesion of multiple random miliary white foci of necrosis in the chinchilla.[6] Conference participants discussed

the differential diagnosis for the macroscopic finding of miliary white foci in the liver of rodents, which includes: *Yersinia pseudotuberculosis*, *Yersinia pestis*, *Francisella tularensis*, *Escherichia coli*, and group b *Streptococcus spp*, among others.

Conference participants also noted multifocal moderate macrovesicles within hepatocytes that often peripheralize the nuclei, interpreted as lipid vacuolar degeneration. This animal had a reported clinical history of inappetence for four days prior to death. Gross photographs document abundant mesenteric and subcutaneous fat



Liver, chinchilla: A tissue Gram stain demonstrates the presence of numerous gram-positive bacilli within necrotic foci. (Gram, 100X) (Photo courtesy of: Department of Veterinary Pathology, Faculty of Veterinary Medicine University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia. http://www.vef.unizg.hr/)

stores. Anorexia in this animal likely caused excessive delivery of free fatty acids from abundant adipose tissue. Excessive delivery of fatty acids, in conjunction with hepatocyte damage from the bacterial infection, likely impaired synthesis and secretion of lipoproteins leading to excessive accumulation of triglycerides in hepatocytes.[9]

Listeria monocytogenes was first described in 1926 by microbiologist Dr. Everitt G. Murray based on six cases of sudden death in young rabbits. He described it as a disease that causes an infiltration of large mononuclear leukocytes and named it *Bacterium monocytogenes*.[5] The bacterium was renamed in 1940 to *Listeria monocytogenes* in honor of famed surgeon and pioneer of antiseptic technique, Dr. Joseph Lister. Interestingly, *Listeria monocytogenes* was not identified as a foodborne pathogen until 1981.[4]

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caused by a hitherto undescribed bacillus Bacterium monocytogenes. *J Pathol Bacteriol*. 1926; 29:407–439.
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CASE II: 11-106289 (JPC 4003035).

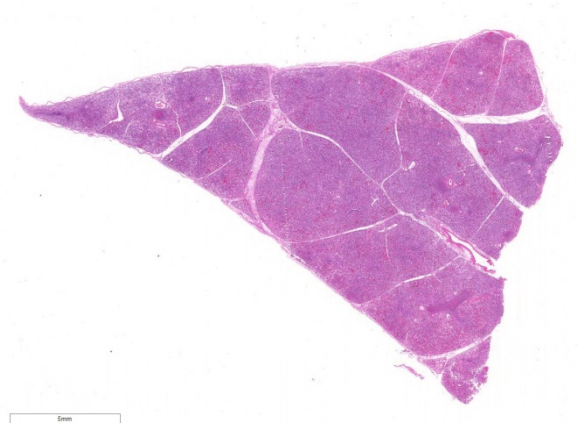
Signalment: One-month-old female Holstein calf (*Bos taurus*).

History: This animal had diarrhea complicated with pneumonia.

Gross Pathology: Presented was a severely thin animal with no body fat. A catheter tube was inserted on the left dorsal-lateral back, tunneled through subcutaneous, and ended at the left side of stomach. The spleen was markedly enlarged with prominent white pulp. All peripheral lymph nodes were four to six times enlarged. This animal had mild to moderate thymic and muscular atrophy.

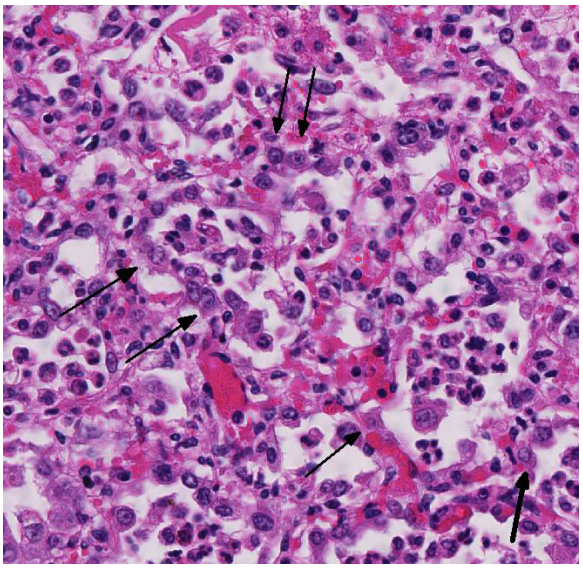
Laboratory results:

Lung PCR positive for bovine respiratory syncytial virus
Lung PCR positive for *Mycoplasma bovis*
Lung IHC positive for bovine respiratory syncytial virus
Aerobic culture of lung – no respiratory pathogens isolated



Lung, calf: At subgross magnification, the lung is diffusely consolidates, airways are full of a cellular exudate, and intralobular septa are diffusely expanded by edema and clear space. (HE, 4X)

Histopathologic Description: Diffusely, alveoli contain moderate numbers of neutrophils and macrophages, along with sloughed type II pneumocytes and occasional syncytial cells, and variable fibrin and erythrocytes. Bacteria are present in alveoli in some sections. Alveolar septal walls are variably lined by type II pneumocytes. Bronchi contain large numbers of neutrophils, sloughed cells, and syncytial cells. Bronchiolar epithelium varies from columnar to cuboidal or occasionally flattened, and intracytoplasmic eosinophilic inclusions are sometimes present in bronchiolar epithelial cells and syncytial cells. Occasional syncytial cells are present in the bronchiolar epithelium. Scattered larger bronchioles and bronchi often are filled with necrotic cells and debris and degenerating neutrophils. Interlobular septae are distended by edema fluid.



Lung, calf: Diffusely alveolar spaces are filled with low to moderate numbers of neutrophils and fewer macrophages, and alveolar septa are lined by patchy Type II pneumocyte hyperplasia (arrows). (HE, 400X)

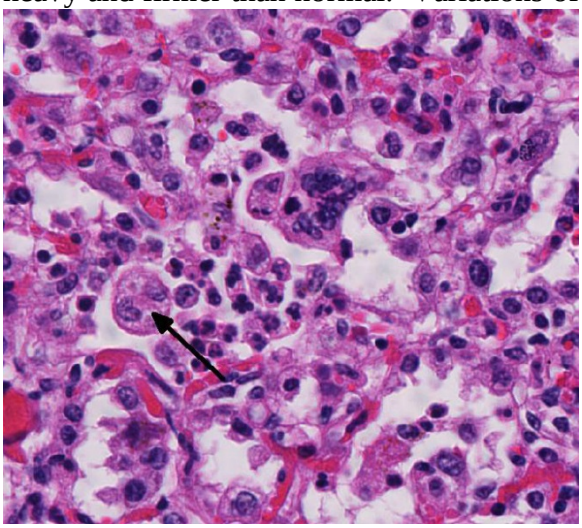
Contributor's Morphologic Diagnoses 1. Lung: Pneumonia, interstitial, proliferative, with syncytial cells and neutrophilic and histiocytic alveolitis, and intracytoplasmic viral inclusions
2. Lung, bronchi and bronchioles: Intraluminal necrotic exudate

Contributor's Comment: The proliferative interstitial pneumonia in this case is attributed to infection with bovine respiratory syncytial virus (BRSV). The necrotic exudate that is present in the bronchi and to a lesser extent in the bronchioles is likely associated with co-infection with *Mycoplasma bovis*.

BRSV is a cause of "enzootic pneumonia" in 2-week to 5-month-old calves with a peak incidence at 1-3 months of age. BRSV can also cause fatal bronchointerstitial pneumonia in feedlot cattle but a role of BRSV in acute interstitial pneumonia in the late feeding period is questionable.[1]

BRSV is a member of the *Pneumovirus* genus in the family *Paramyxoviridae*. Experimental infections result in less severe clinical disease than natural cases, attributed to a reduction in virulence as a result of in vitro passage of the virus. In natural infections, viral antigen can be located in bronchiolar epithelium, type II pneumocytes, and macrophages, and less frequently in the nasal, tracheal, and bronchial epithelium.[1]

Gross lesions of natural BRSV infection differ in cranioventral and caudodorsal areas of the lung. The cranioventral lung is atelectatic, collapsed, deep red or mottled, and rubbery. In contrast, the caudodorsal areas fail to collapse and are edematous, heavy and firmer than normal. Variations of the gross lesions do occur, such that some cases may have a generalized rubbery texture with no difference between cranial and caudal lung, and in some cases there may be formation of bullae.[1]



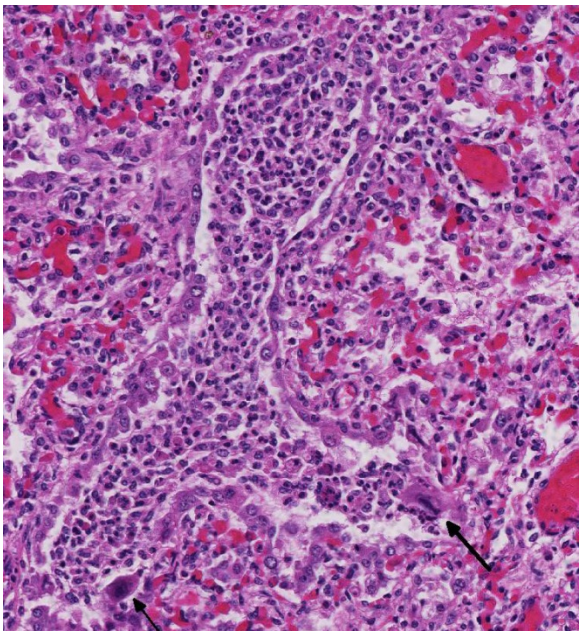
Lung, calf: Alveoli contain multinucleated viral syncytia which often contain intracytoplasmic viral inclusions (arrow). (HE, 400X)

Microscopic lesions of BRSV pneumonia include bronchointerstitial pneumonia with necrotizing bronchiolitis, formation of bronchiolar epithelial syncytia, and exudative or proliferative alveolitis. In acute lesions, bronchioles are lined by flattened

epithelium, bronchiolar lumens contain necrotic epithelial cells and neutrophils. Alveoli contain neutrophils and macro-phages; hyaline membranes are in-frequent. Syncytia may be difficult to distinguish from the multinucleate macro-phages that clear fibrin from alveoli in cases of fibrinous pneumonia, however, the presence of bronchiolar syncytia is a more reliable indicator of viral infection. Intracytoplasmic eosinophilic inclusion bodies are occasionally present in syncytial cells and uncommonly in bronchiolar and alveolar epithelium.[1]

BRSV infections in calves have many similarities to human RSV infections. Children vaccinated with formalin-killed RSV vaccine reportedly had more severe respiratory disease than those who were not vaccinated.[3] Similarly, there are reports of exacerbated disease after vaccination of cattle with formalin-killed vaccine. Kalina et al[5] showed that vaccination with formalin killed vaccine can enhance the disease, and attributed this to a shift to a Th2 immune response. Experimental infection of calves with BRSV, followed by infection with *Histophilus somni* resulted in an infection that was more severe than *Histophilus* alone; this was attributed to a shift to IgE production by the BRSV infection.[4] Current trends are to develop vaccines for BRSV that shift the immune response to a Th1 response that will favor virus-killing mechanisms.

The calf in this study was co-infected with *Mycoplasma bovis*, and the necrotic cells and necrotic debris in the airways are attributed to *M. bovis* infection. The mycoplasmal infection in this calf probably is in early stages of development, since the necrotic lesions have not progressed beyond the airways. The early lesions of experimental *M. bovis* infection consist of

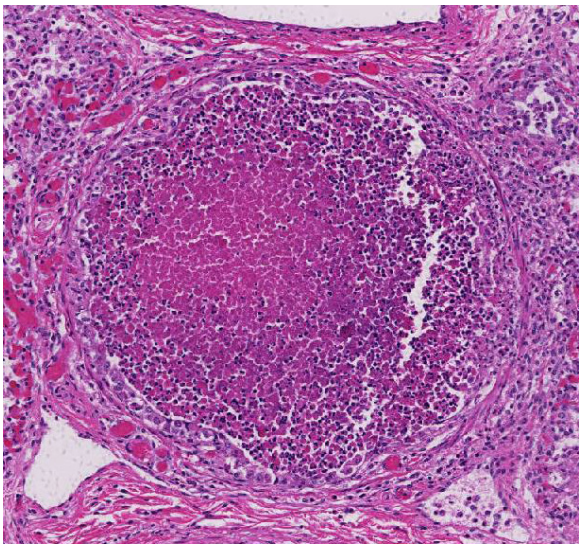


Lung, calf: The lumina of bronchioles are filled with large numbers of viable and degenerate neutrophils; bronchiolar epithelium is multifocally necrotic, segmentally attenuated or hypertrophic, and there are several viral syncytia within the population (arrows). (HE, 400X)

suppurative exudates within small bronchioles, in which the leukocytes are stated to have a characteristic appearance-they are necrotic but retain their cellular outlines, and have hypereosinophilic cytoplasm, and unapparent or fragmented nuclei.[1]

JPC Diagnosis: Lung: Pneumonia, bron-chointerstitial, necrotizing and histiocytic, subacute, diffuse, severe, with type II pneumocyte hyperplasia, syncytial cells, and epithelial intracytoplasmic viral inclusion bodies, Holstein, *Bos taurus*.

Conference Comment: This case generated intense discussion among conference participants regarding the pattern of inflammation present within the section of lung. The morphologic patterns of pneumonia can be classified based on the initial site of involvement and the spread of the lesion. In general, there are four



Lung, calf: The exudate of several bronchioles contains an exudate undergoing lytic necrosis, suggestive of mycoplasmal infection. (HE, 400X)

morphologic patterns of lung disease: airway disease involves inflammation and necrosis targeting bronchi and bronchioles; bronchopneumonia involves inflammation and necrosis of alveoli and bronchioles due to due to aerogenous bacteria less than 5 μm in diameter; interstitial pneumonia arises from disease of the non-airway tissues, such as alveolar and interlobular septa; and bronchointerstitial pneumonia is a combination of airway and interstitial lung disease.[1] As mentioned by the contributor, typically bovine respiratory syncytial virus (BRSV) causes bronchointerstitial pneumonia with necrotizing bronchiolitis, type II pneumocyte hyperplasia, exudative or proliferative alveolitis, and syncytial cell formation.[1,5] In this case, there is severe necrosis of the bronchiolar epithelium. This is in contrast with bronchopneumonia, where bronchioles and alveoli are filled with leukocytes as a result of bacterial infection of the airways, but necrosis of the epithelium is usually not present.

Like the contributor, some conference participants favored breaking the pattern of inflammation into two distinct processes. Participants argued that BRSV causes a fibrinous and proliferative interstitial pneumonia with a secondary suppurative and necrotizing bronchopneumonia caused by early co-infection with *Mycoplasma bovis*, detected by PCR in this case. Participants favoring the pattern of bronchointerstitial pneumonia countered that the virus causes the necrotizing lesions in the bronchi, bronchiolar epithelium, and type I pneumocytes, as well as thickening of the alveolar septae by infiltrates of leukocytes, and type II pneumocyte hyperplasia. They also point out that there is little evidence for typical *M. bovis* lesions in this case. *M. bovis* classically causes a bronchopneumonia with distention of bronchioles and alveoli by a caseonecrotic exudate with an eosinophilic core, and are rimmed by "ghost-like" leukocytes remnants.[1] Most conference participants agreed that those lesions associated with *M. bovis* are not a prominent feature of this case. Ultimately, participants agreed that the lesions in this case are characteristic for BRSV, which causes bronchointerstitial pneumonia in the cranio-ventral lung lobes.[1,2,5]

Conference participants also discussed additional causes of syncytial cells in the bovine lung, other than BRSV. Bovine parainfluenza virus 3 (BPIV-3), a member of the genus *Respirovirus* in the *Paramyxoviridae* family, can also induce the formation of syncytia with intracytoplasmic inclusion bodies.[1] Similar to BRSV, another *Paramyxoviridae* family virus, BIPV-3 induces syncytial cell formation via a fusion (F) transmembrane glycoprotein.[1,5] BIPV-3 usually causes mild bronchitis and bronchiolitis in cattle. Multinucleated alveolar macrophages can also resemble syncytial cells in cattle with fibrinous or granulomatous pneumonia. However, in BRSV, there would likely be both alveolar and bronchiolar syncytial cells.[1]

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CASE III: 13A888 (JPC 4066311).

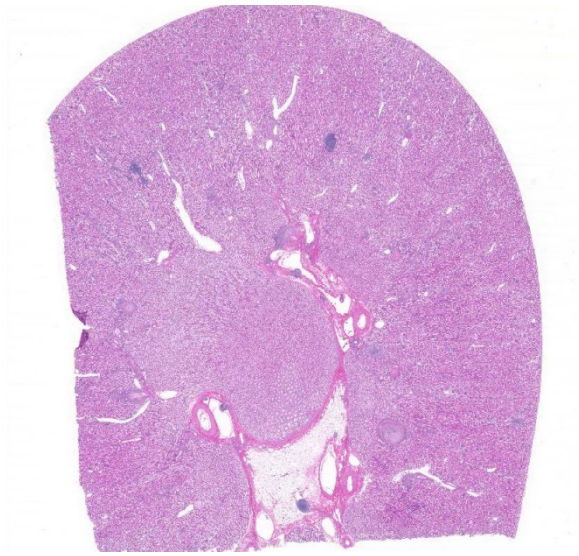
Signalment: Five-year-old male rhesus macaque (*Macaca mulatta*).

History: This animal was assigned to the research project of alcohol, HIV infection & host defense. Ethanol was administered via the gastric catheter with 30% ethanol in water (w/v) as a 0.5-hour prime, followed immediately by a 4.5-hour maintenance infusion. The concentration of ethanol in blood was 50 to 60 mM. The animals received ethanol four consecutive days per week for the duration of the study. Three months after ethanol administration, this animal was intravenously inoculated with simian immunodeficiency virus (SIV) 251 about one year before sacrifice. *Streptococcus pneumoniae* was inoculated in right lung seven months before sacrifice.

Six months after SIV inoculation, this animal began to show chronic, mild leukocytosis, mild neutrophilia, and moderate thrombocytopenia. The animal developed weight loss, loss of muscle mass, enlargement and restriction of stifles, enlarged lymphoid tissue, and mild splenomegaly and hepatomegaly.

Gross Pathology: Presented was a severely thin animal with no body fat. A catheter tube was inserted on the left dorsal-lateral back, tunneled through subcutaneous, and ended at the left side of stomach. The spleen was markedly enlarged with prominent white pulp. All peripheral lymph nodes were four to six times enlarged. This animal had mild to moderate thymic and muscular atrophy.

Laboratory results: N/A

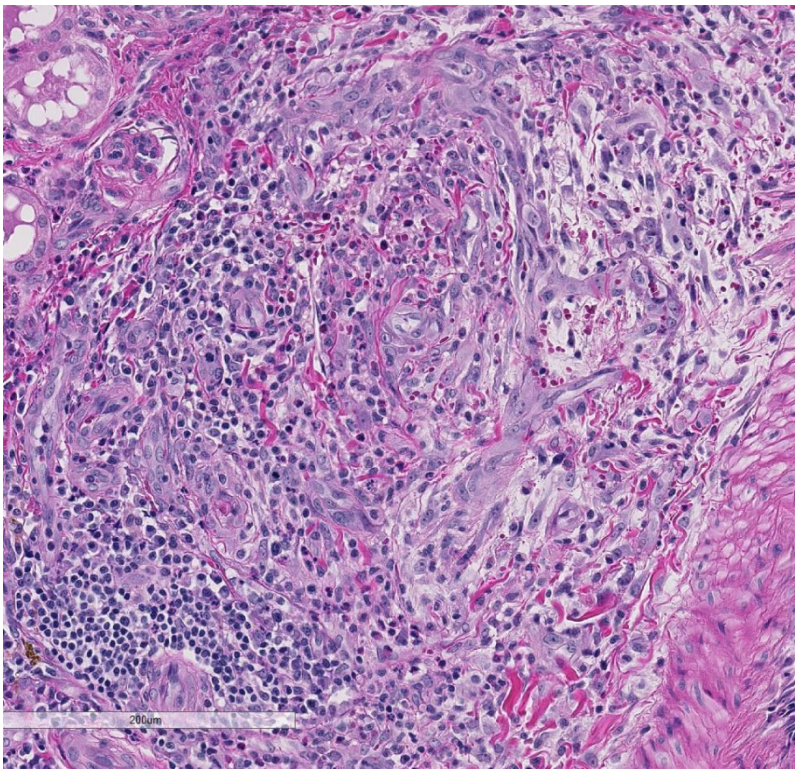


Kidney, macaque. At subgross magnification, there are lymphoid nodules scattered throughout the section, and several vessels, predominantly arcuate arteries at the corticomedullary junction, are expanded and surrounded by a cellular infiltrate. (HE, 5X)

Histopathologic Description: Kidney: Multifocally the small and medium-sized arteries are expanded and variably disrupted by proliferation of the tunica intima, smooth muscular hyperplasia, and infiltration of inflammatory cells. The lumina of affected arteries are partially to completely occluded and lined by hypertrophic endothelial cells. Usually, the tunica media are segmentally to circumferentially thickened by smooth muscle hyperplasia, fragmented collagen bundles, and reactive fibroblasts. The tunica adventitia is markedly expanded by numerous neutrophils, lymphocytes, plasma cells, and fewer macrophages and eosinophils. Some subendothelial tunica intima and tunica media are disrupted and markedly expanded by thick bands of deeply eosinophilic hyaline to fibrinoid material admixed with cellular and karyorrhectic debris and many erythrocytes (necrosis and hemorrhage). Multifocally the renal tubules are ectatic, lined by attenuated epithelial cells, and contain hypereosinophilic homogenous material (protein casts) and cellular debris. Multifocally the interstitium is infiltrated by many lymphocytes and plasma cells. Occasionally the interstitium is expanded by lymphoid aggregates (lymphoid hyperplasia and dysplasia). Some glomerular tufts are senescent and shrunken with ectatic Bowman's spaces.

Contributor's Morphologic Diagnoses: 1. Kidney, medium and small arteries: Arteritis, proliferative and necrotizing, multifocal.
2. Kidney, lymphoplasmacytic interstitial nephritis, multifocal, mild.
3. Kidney, interstitial lymphoid hyperplasia and dysplasia.

Contributor's Comment: Arteriopathy is also noted in the small to medium arteries of the mesentery, testis, liver, gall bladder, pancreas, urinary bladder, and bone marrow. The systemic vascular lesions in this monkey resemble those found in polyarteritis nodosa (PAN)-like syndrome in HIV patients. PAN-like syndrome has been described in HIV patients in the literature.[6,7] While target organs are usually muscles, nerves, skin and gastrointestinal tract, renal polyarteritis nodosa in HIV patients has also been reported.[2] PAN-like syndrome occurs in fewer than 1% of HIV patients. The underlying mechanism is thought to involve cell or immune-complex-mediated inflammation, like classic PAN in other species. Although the histopathological changes are similar between two entities, there are several important differences between PAN in HIV patients and so-called classic or idiopathic PAN. First, the waxing and waning clinical course of classic PAN is not seen in patients with HIV infection. Second, classic PAN can be associated with hepatitis B virus infections, but in HIV patients, the serology for HBV is always negative. Third, the affected arteries in HIV-associated PAN tend to be smaller than that seen in classic PAN.[4]

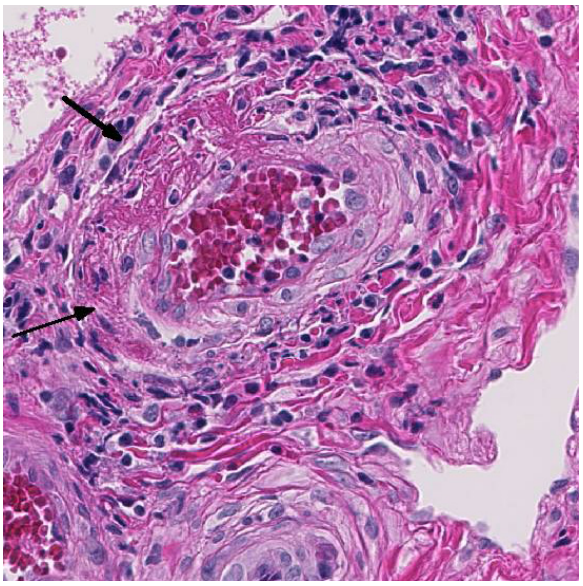


Kidney, macaque. Arcuate arteries and branches are often replaced by a large mass of tortuous vessels with prominent endothelium. The adventitia is expanded by low to moderate numbers of lymphocytes and plasma cells, and fewer macrophages. (HE, 320X)

Pulmonary arteriopathy is the most common vasculopathy in macaques infected with SIV. Nineteen of 85 animals infected with SIV developed pulmonary arteriopathy characterized by intimal thickening, luminal occlusion, and internal elastic laminae fragmentation and interruption.[3] Pulmonary artery hyperplasia and/or plexiform arteriopathy were present in eight of 13 (62%) SHIV-infected macaques.[5] However, the pulmonary arteries were histopathologically normal in the current case. This observation is consistent with the two published cases, in which, arteriopathy was mild or absent in the lungs.[11] These observations suggested a different pathogenesis between pulmonary arteriopathy and PAN-like syndrome in SIV infected monkeys.

PAN-like syndrome has been reported in two SIV-infected rhesus macaques.[11] Vasculopathy is prominent in kidney, intestine, pancreas, liver, heart, lymph nodes, spleen, and testis. Histologically, disseminated arteriopathy is characterized by intimal thickening and fibrosis with varying degrees of vasculitis. Intranuclear inclusion bodies were CMV positive by immunohistochemistry in multiple organs in these two monkeys. Intranuclear inclusion bodies were not observed in the current case but immunohistochemistry for CMV or other viral agents was not performed in the current case.

Based on extensive experience on this model, it is unlikely that ethanol administration was associated with PAN-like syndrome in this monkey. There is no documentation of alcohol and arteriopathy in the literature. Although this animal was inoculated with Streptococcus pnenumonae, grossly and microscopically there was no current evidence of Streptococcus infection. Renal interstitial lymphoid hyperplasia and dysplasia are not uncommon findings in SIV-infected monkeys.



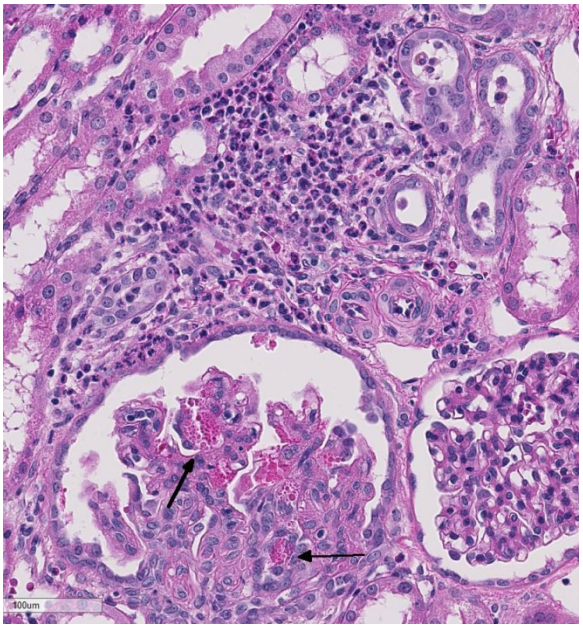
Kidney, macaque. There is transmural hyaline change within the wall of smaller arterioles as a result of extrusion of protein from the lumen. (arrows) (HE, 320X)

JPC Diagnosis: 1. Kidney, small arteries and arterioles: Arteriopathy, proliferative and necrotizing, multifocal, mild to marked, with adventitial inflammation, rhesus macaque (*Macaca mulatta*).
2. Kidney: Interstitial nephritis, lympho-plasmacytic, multifocal, mild.

Conference Comment: Although the specific etiology and pathogenesis of this lesion are unclear, the contributor provides an excellent example of a polyarteritis nodosa (PAN)-like syndrome in a non-human primate. PAN-like syndromes are thought to be a type III hypersensitivity reaction secondary to antigen:antibody complex deposition in medium to small caliber arteries.[1] Immune complex deposition results in complement activation leading to segmental, circumferential, and proliferative arteritis. This syndrome has been well described in the aged Sprague-Dawley rat and beagles.[9,10] In rats, lesions most often occur in the muscular medium-sized arteries of the mesentery, pancreas, testis, hepatic, coronary, uterine, cerebral, adrenal, and renal arteries.[10] This condition in beagles is associated with beagle pain syndrome. In these cases, the coronary and meningeal arteries are most affected, and clinically dogs are febrile, lose weight, and have cervical pain.[9] Typically in domestic species, PAN-like syndrome spares the pulmonary circulation, large arteries and glomeruli.[11] The association of SIV as part of the pathogenesis of the arteriole lesions, in this case, remains unclear.

The JPC strives to avoid using the suffix "-opathy" in a morphologic diagnosis due to its non-specific nature; however, in rare instances, this terminology may be appropriate, especially in cases where the primary process underlying the lesion is difficult to ascertain. While the SIV-positive status of this particular animal suggests a causal relationship, PAN has also been seen as a spontaneous finding in macaques, as well as a toxic lesion association with administration of cyclosporine and tacrolimus (WSC 2003-2004, Conference 20, Case 1). The term arteriopathy can be modified by other descriptors such as proliferative and necrotizing to further define the underlying process. Much of the literature on this disease uses the term arteriopathy to describe this finding in arteries and arterioles in SIV-infected rhesus macaques.

During a discussion of the vessel wall changes in this case, some conference participants preferred the term hyaline change to describe the circumferential homogenous, eosinophilic, proteinaceous material deposited within the external elastic membrane of arterioles rather than the well-ensconced term fibrinoid necrosis. Fibrinoid necrosis has been classically used by both human and veterinary pathologists to describe the brightly eosinophilic changes in the injured vessel associated with immune complex, plasma protein, and complement



Kidney, macaque. There are multiple foci of eosinophilic inflammation scattered throughout the interstitium; tubules within these areas are mildly atrophic. Within the adjacent glomerular tuft, there is extrusion of protein into the mesangium (arrows)

protein deposition within vessels.[1] Fibrinoid necrosis implies a pathogenesis that may or may not be present. The brightly eosinophilic homogenous protein accumulation obscures the structural detail of the blood vessel, thus making it difficult or impossible to determine if there is fibrin or necrosis present within the arteriole wall. Hyalinosis describes the accumulation of leaked eosinophilic proteinaceous material secondary to endothelial damage and increased vascular permeability without making assumptions about the pathogenesis.

Finally, several participants found multinucleated giant cells within the tubular epithelium and lumina of collecting ducts within their sections. Multinucleated giant cells have been described as a common incidental finding in macaques[8]; however, a number of participants ascribed them as a potential corroborating sign of lentivirus infection in this macaque.

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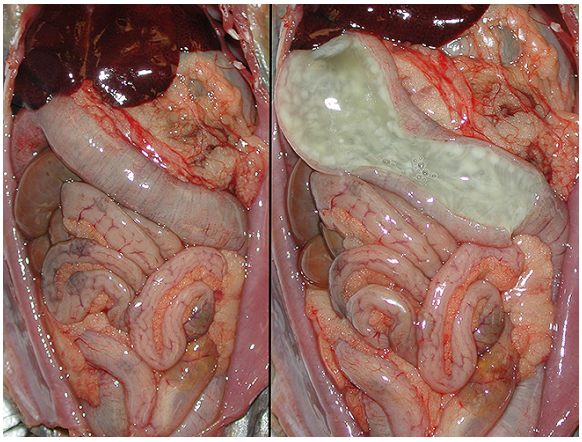
CASE IV: CHIN1 (JPC 4084953).

Signalment: Adult female common marmoset (*Callithrix jacchus*).

History: This marmoset was one of a cohort in quarantine that had a history of intermittent diarrhea of undetermined origin. There had been a brief response to Baytril therapy. Several animals had then been used for terminal experimental manipulation subsequent to their rapid clinical deterioration.

Gross Pathology: The entire large bowel, cecum through rectum, was dilated and had a swollen, edematous appearance from the serosal surface. On opening, the bowel lumen was filled with large amounts of clear, thick gelatinous material. Underlying this content, there was an irregular pattern of coalescing cream-colored nodularity. This superficial mucoid material was adhered to remaining underlying mucosa, although it

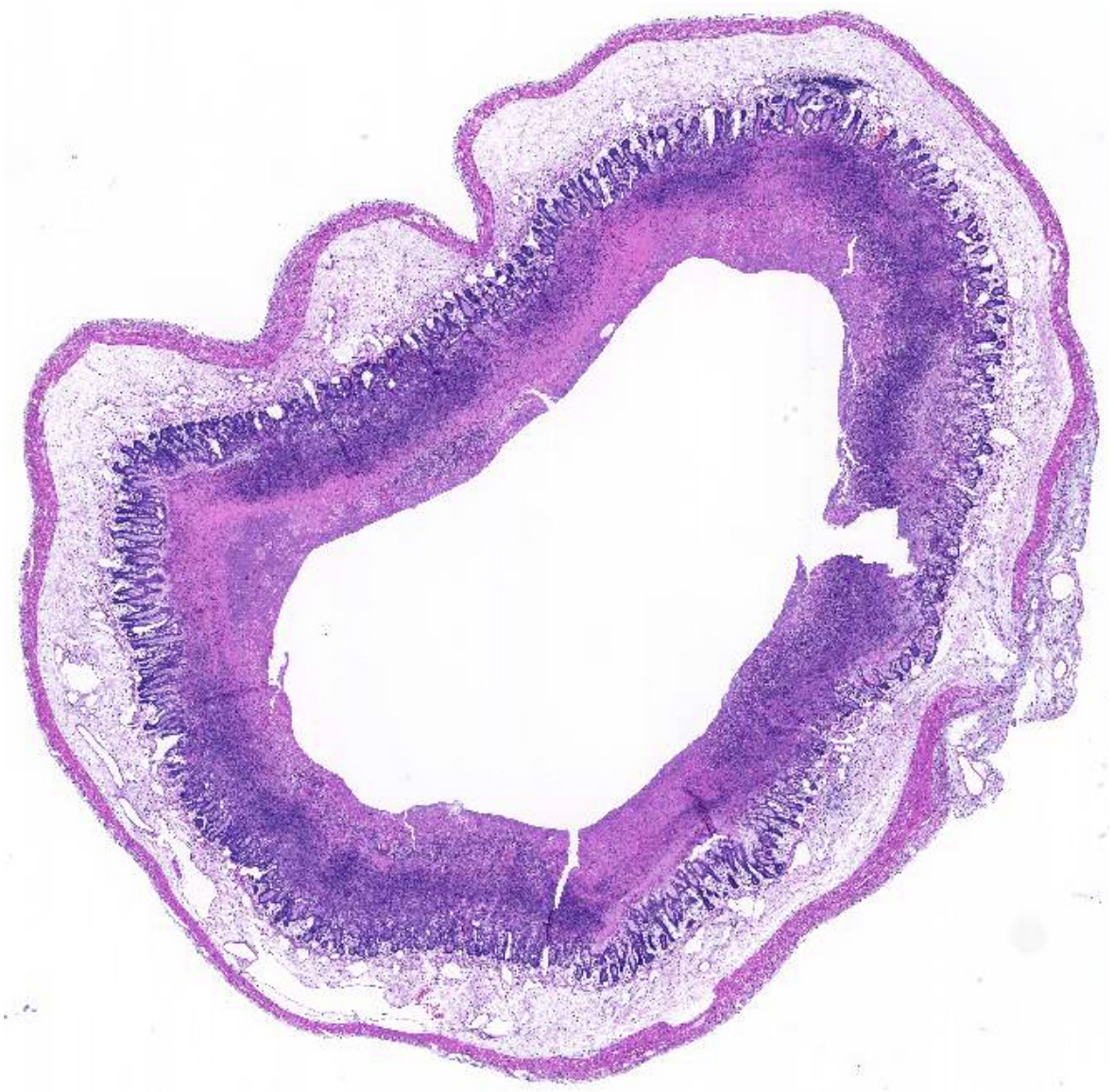


Colon, marmoset: The colon is markedly distended and filled with a clear gelatinous material. Nodular whitish areas of necrosis are present within the underlyingmucosa. (Photo courtesy of: University of Pittsburgh, Division of Laboratory Animal Resources, http://www.dlar.pitt.edu/)

was possible through blunt dissection, to "peel" this off as a layer. Minimal fecal content was present within the large intestine. Stomach and small intestine were also largely empty with the exception of a very small amount of semi-solid ingesta in the distal ileum.

Laboratory results: N/A

Histopathologic Description: Full-thickness pieces were evaluated from different segments of the colon. All were similar in appearance. Diffuse necrosis of the superficial mucosa was present with an associated thick exudative layer of fibrin, mucin, leukocytes (primarily neutrophils) and nuclear debris. Patchy basophilic aggregates of bacterial colonies including numerous large rod-shaped organisms were

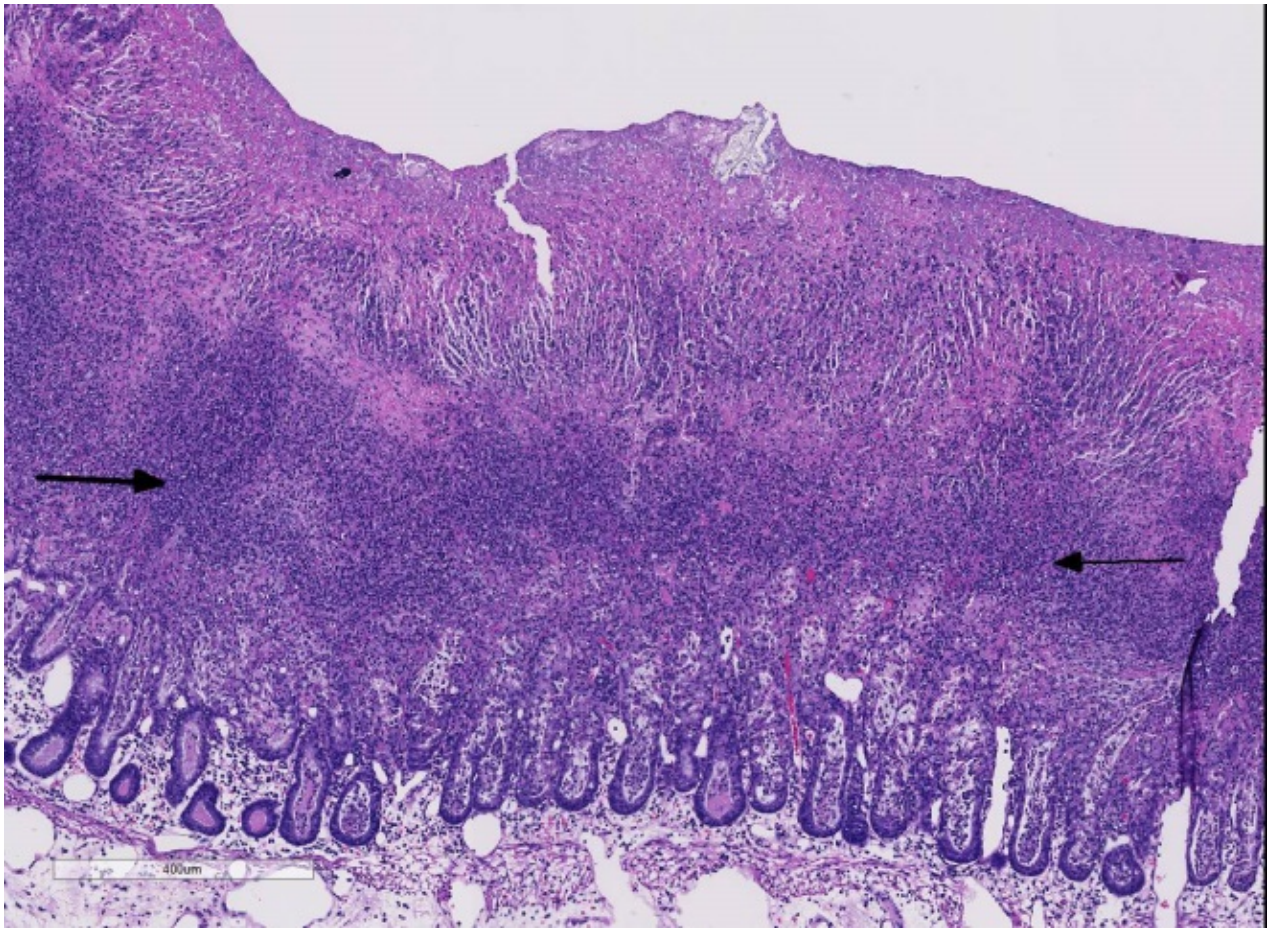


Colon, marmoset. The mucosa is covered with a fibrinonecrotic membrane which is border on its deepest aspect by a dense band of basophilic cellular debris. There is marked edema of the submucosa, as well as edema in the lamina propria and serosa. (HE, 5X)

scattered throughout the overlying necrotic membrane. In many areas, a characteristic striated pattern formed by columns of PMN cells and mucin was present. Inflammatory infiltrates extended into deeper intact mucosal crypt structures, which also often contained abundant mucinous debris. The submucosa (and to a much lesser extent, muscularis) was markedly expanded by edema & mild scattered inflammatory infiltrates and had prominently dilated vascular and lymphatic vessels.

Contributor's Morphologic Diagnosis: Colitis, fibrinonecrotizing and pseudo-membranous, diffuse, severe, with extension of inflammation into crypts and marked submucosal edema.

Contributor's Comment: The gross and microscopic findings in this case are consistent with *Clostridium difficile* colitis, also referred to as *C. difficile*-associated disease (CDAD). Fecal content submitted from this and other animals in the cohort



Colon, marmoset. A dense band of cellular debris is present in the middle of the mucosa as delimiting the leading edge of the diffusing exotoxin of C. difficile (arrows), and resulting mucosal necrosis. Subjacent colonic glands are distended and filled with cellular debris. (HE, 45X)

was PCR-positive for *C. difficile* Toxin B gene.

C. difficile is a confirmed pathogen in humans and a wide variety of mammals. Infection is a well-recognized clinical entity in horses, pigs, hamsters and rabbits.[6] Disease in horses occurs in both adults and foals, with both antibiotic treatment and hospitalization being clear risk factors. Gross and microscopic lesions may not be sufficient to distinguish CDAD from other causes of acute equine enteric disease, including those associated with other clostridial pathogens.[3] Conversely, primarily neonatal pigs appear susceptible to infection – usually around 5 days of age.[14] Hamsters are the most susceptible animals to natural infection, with as little as 1 colony forming unit leading to disease in antibiotic pretreated animals.[7] Although *C. spiriforme* is a more common pathogen, *C. difficile* does cause peracute death without clinical signs in rabbits.[2]

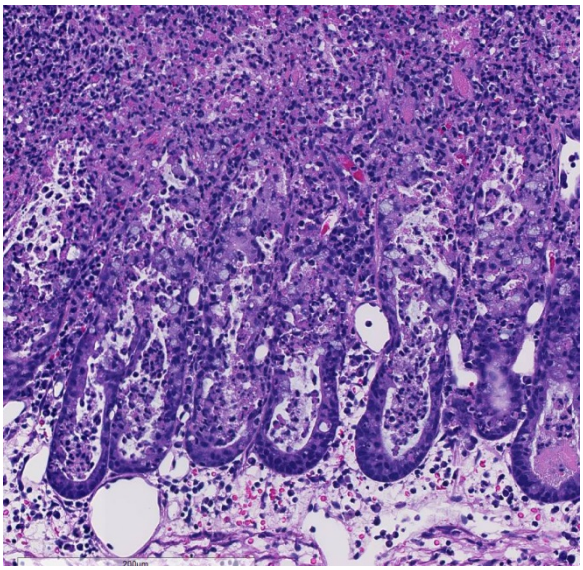
Reports of natural or experimental infection exist in many other species including guinea pigs, mice, rats, domestic cats and dogs, calves, ostriches, prairie dogs and non-human primates.[6,12] This is to the contributor's awareness, the first documented cases of the entity in this primate species (marmoset).

Although the cecum and colon are the sites most often affected in the majority of

species, the nature of lesions present, their location/distribution, and age of host susceptibility all vary significantly. Specifically, foals and rabbits consistently develop severe lesions in the small intestine.[6]

C. difficile is recognized as one of the most important nosocomial pathogens in humans, causing illness ranging from mild diarrhea to fulminant colitis. Antibiotic use and hospitalization are significant risk factors, with recurrence being common (15-20% of cases). The disease has been well characterized histologically, occurring in three stages: 1) focal epithelial necrosis along with fibrin-rich exudates, 2) marked exudation protruding through an area of mucosal ulceration (characterized as classical "volcano" lesions), and 3) diffuse, more severe mucosal ulceration with necrosis and associated pseudomembrane composed of fibrin, leukocytes and cellular debris. In cases where disease has progressed to pseudomembrane formation, endoscopy can be diagnostic, and although a rapid means of testing, it is invasive and expensive.[10]

C. difficile infection occurs when the natural flora in the gut is disrupted.[9] Although this is often associated with antibiotic administration, in some species, stress, change of diet, transportation, starvation, and medical or surgical treatment can initiate disease.[3] After oral ingestion, spores, which are resistant to the acidity of the stomach, germinate into the vegetative form in the small intestine, leading to toxin production resulting in colitis.[4] Other factors such as host susceptibility or virulence factors of the infecting strain may also be integral in determining the clinical outcome of infection. Both toxins, TcdA and TcdB, are cytotoxic, causing disruption of the actin cytoskeleton and tight junctions, resulting in decreased transepithelial resistance, fluid accumulation and destruction of the intestinal epithelium. These substances also cause the release of various inflammatory mediators from enterocytes, mast cells and macrophages.[9] In humans, host response appears critical and protective in the clinical outcome, with patients having elevated levels of IgG and IgA against toxin A.[13]

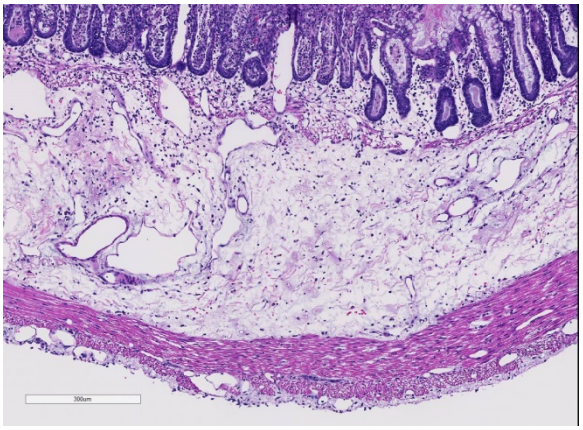


Colon, marmoset. Higher magnification of the affected colon glands, which are ectatic and filled with variable amounts of necrotic enterocytes, cellular debris, and degenerate neutrophils (crypt abscesses) (HE, 140X)

The detection of *C. difficile* by culture is rarely performed for diagnostic purposes because of the slow turnaround time.[5] Rather, identification of *C. difficile* toxins in the stool using ELISA methods are commonly used. These have excellent specificity, but variable sensitivity (75-85%). PCR methods to detect the toxin A and B genes responsible for the production of virulence factors are available (Cleveland Clinic paper synopsis).

Although infection and disease in man and animals has long been considered nosocomial and/or antibiotic related, recent

studies have explored whether zoonotic and foodborne transmissions occur. Certainly significant percentages of both companion and food animals are known to carry *C. difficile*. Although there are no scientific reports explicitly confirming that *C. difficile* can be acquired via foods or contact with animals, there is sufficient laboratory and epidemiological data to suggest that this may occur and that interventions to prevent transmission should be adopted.[5,11]



Colon, marmoset. There is marked edema and lymphatic dilation within the submucosa, which is less severe than that seen in the lamina propria and serosa. (HE, 60X)

The specific source of the infection in these marmosets was not determined, nor was the role of the fluoroquinolone treatment used in their initial empirical therapy (though this class of antimicrobials may pose a greater risk for development of disease than others).[4] This case does underscore the importance of awareness by both clinicians and pathologists of the potential for antibiotic-associated diarrheal diseases to occur in multiple species.

JPC Diagnosis: Colon: Colitis, necrotizing, circumferential, diffuse, severe, with fibrinocellular pseudomembrane and marked submucosal edema.

Conference Comment: The contributor provides an outstanding review of general information, diagnosis, pathogenesis, gross and microscopic lesions, and epidemiology of pseudomembranous colitis caused by *Clostridium difficile* across many species.

Conference participants noted the presence of a prominent circumferential fibrino-necrotic membrane replacing the apical mucosal epithelium, with a sharp line of demarcation separating necrotic tissue from relatively unaffected crypt epithelium. Also, within ulcerated areas this case nicely demonstrates the classic "volcano" lesions of florid fibrinosuppurative exudation through intestinal crypts.[3] As mentioned by the contributor, *C. difficile* elaborates A and B toxins which diffuse into the tissue from the lumen and destroy the apical mucosal epithelium, in addition to causing the marked submucosal edema demonstrated in this case. Necrosis of the deeper mucosal surface and the colonic tunica muscularis has been reported in chronic cases.[3]

In addition to *C. difficile*, conference participants discussed measles virus as a unique differential for enteritis in common marmosets, and other new world monkeys. This highly contagious, aerosolized virus belongs to the genus *Morbillivirus*, in the *Paramyxoviridae* family.[1,8] Marmosets are highly susceptible to measles infection and clinical disease is characterized by severe gastritis and colitis.[1] High mortality has been reported in marmosets infected with natural virus from human contact.[1] Histologic lesions of measles in marmosets include: epithelial necrosis in the stomach, cecum, and colon, with syncytia, and intracytoplasmic viral inclusion bodies in the mucosal epithelium and the gut-associated lymphoid tissue (GALT).[8] Modified live human vaccines against measles have produced active disease in marmosets and are not recommended as a preventative.[1,8]

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