Joint Pathology Center
Veterinary Pathology Services

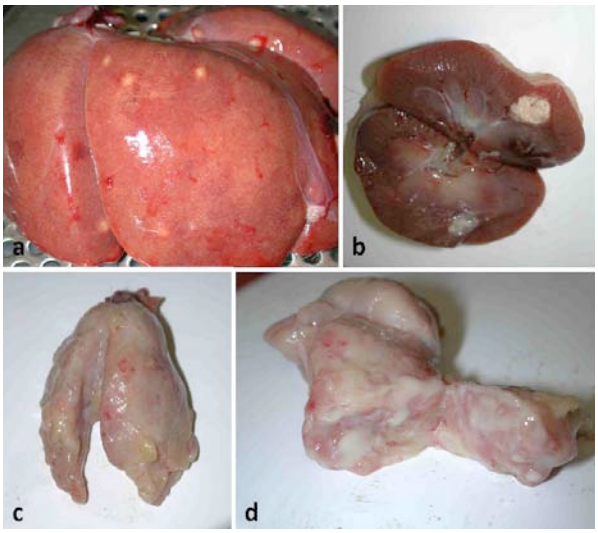
WEDNESDAY SLIDE CONFERENCE 2013-2014

Conference 3

26 September 2013

CASE I: 04135-11 (JPC 4032910).

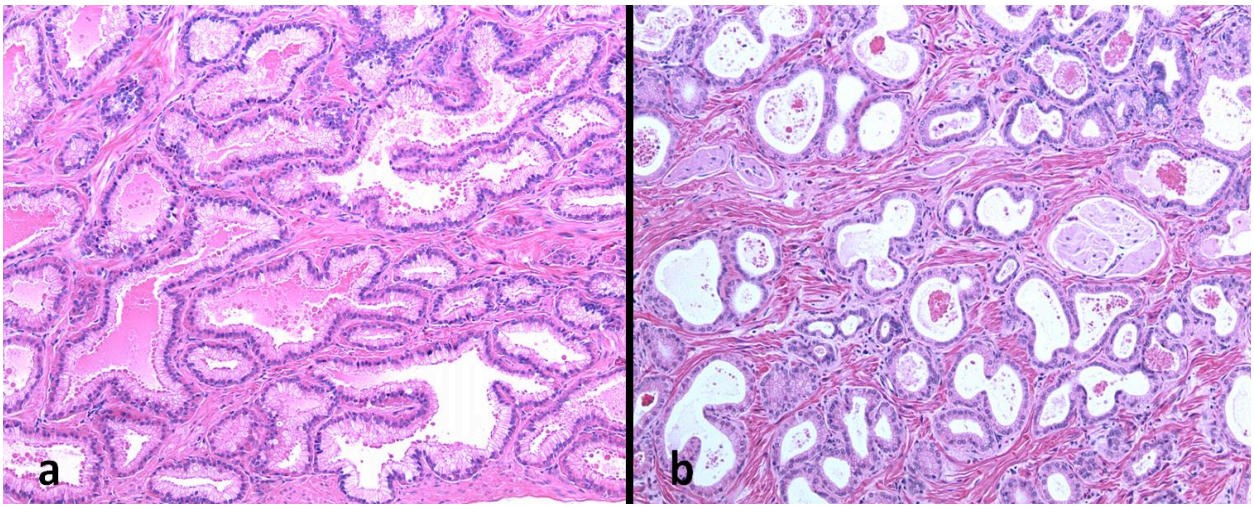
Signalment: 16-year-old male cynomolgus macaque (*Macaca fascicularis*).



1-1. Multiple organs, cynomolgus monkey: a) Variably sized, up to 1 cm diameter, pale green to yellow abscesses were visible on the serosal surface of the liver. b) More caseous foci were present in the kidneys (c, d). The left lobe of the seminal vesicle was enlarged to approximately twice normal owing to extensive abscessation. (Photo courtesy of: Wake Forest University Health Sciences, Animal Resources Program, Medical Center Boulevard, Winston-Salem, NC 27157 http://www.wfubmc.edu/schoolOfMedicine/schoolOfMedicine_default.aspx?id=26651).

History: This animal arrived at Wake Forest University from Indonesia in 2003, and for 1 year was relatively healthy. On the day before death, it presented non-weight bearing on the left rear limb and on the following day was reluctant to move, hypothermic (84.6 F), dehydrated and had mild bradycardia. Radiographs disclosed radiopacity of the left leg proximal to the stifle joint. Blood work revealed anemia (HCT- 22%), a high normal white blood cell count (8,000/ml) with a degenerative left shift, azotemia (creatinine-7.59 mg/dl, BUN- 68 mg/dl), hyperproteinemia (7.5 g/dl), hyperphosphatemia (7.9 g/dl), and mild elevations of the liver enzymes (ALP-311 U/l, GGT-108 U/l). The animal died despite supportive care.

Gross Pathology: A 5.25kg cynomolgus macaque was submitted for necropsy. The subcutaneous tissue surrounding and adjacent to the left tibiotarsal joint was swollen and the joint contained viscous, tan pus. Variably sized, up to 1 cm diameter, pale green to yellow abscesses were visible on the serosal surface of the liver with extension into the parenchyma. Similar lesions were present in the spleen and mesentery. More caseous foci were present in the kidneys. About 2 mL of pus was present in the urinary bladder, and the left lobe of the seminal vesicle was enlarged to approximately twice normal owing to extensive abscessation.



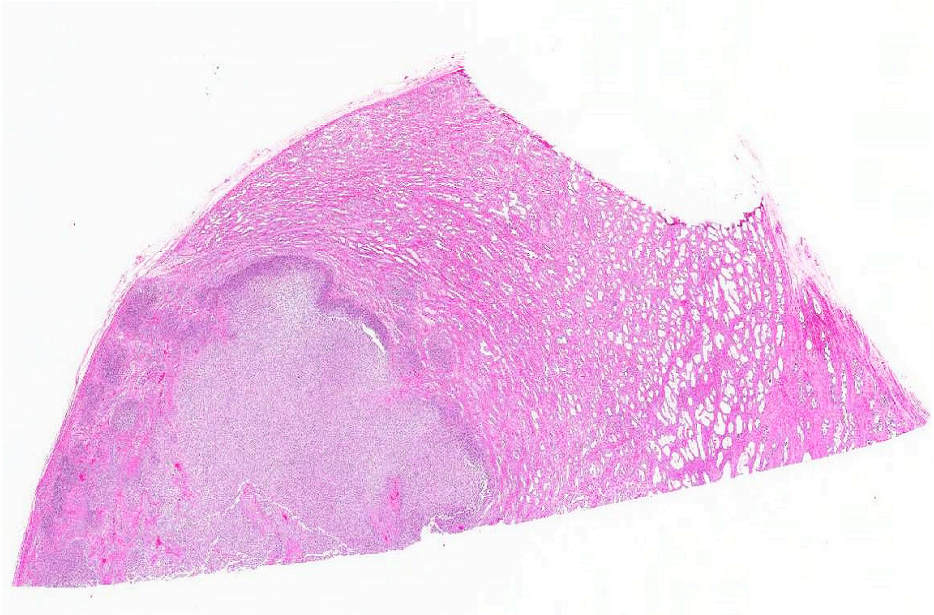
1-2. Prostate gland, cynomolgus monkey: Normal cranial (a) and caudal (b) prostate from a 16-year-old rhesus macaque (Macaca mulatta). The cranial prostate has larger, irregular glands with tall columnar epithelium. The caudal prostate has smaller glands with low columnar to cuboidal epithelial cells. (HE 10X) (Photo courtesy of: Wake Forest University Health Sciences, Animal Resources Program, Medical Center Boulevard, Winston-Salem, NC 27157 http://www.wfubmc.edu/schoolOfMedicine/schoolOfMedicine_default.aspx?id=26651).

Laboratory Results: *Burkholderia pseudomallei* cultured from urinary bladder, and renal and hepatic abscesses.

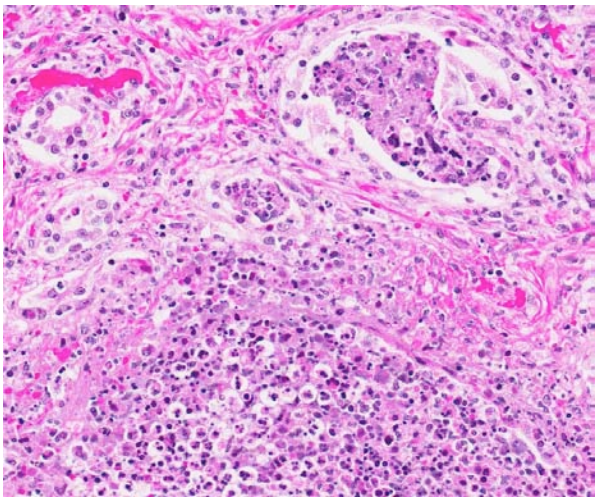
Histopathologic Description: Caudal prostate gland: Extensive abscessation effaces and compresses the glands of the caudal prostate. This reaction is composed of abundant neutrophils, many degenerate, admixed with eosinophilic amorphous material, karyorrhectic debris, fibrin and small accumulations of extravascular erythrocytes (hemorrhage). Adjacent prostatic glands are filled with similar material. In some sections, lymphocytes are present in the prostatic stroma, while in others, there is a suppurative exudate present within the urethral lumen.

Contributor's Morphologic Diagnosis: Prostatitis, multifocal, suppurative, subacute, severe.

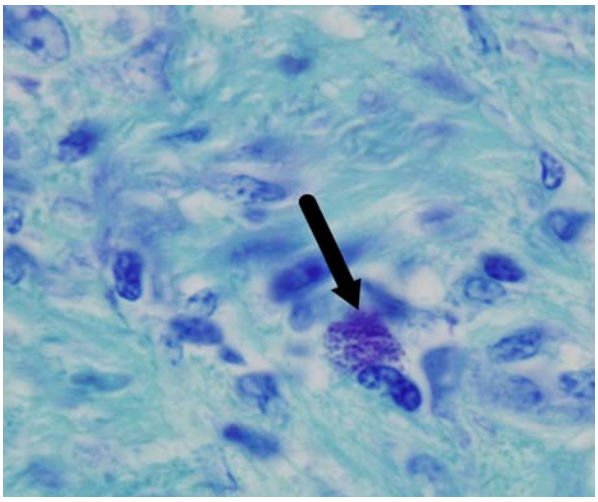
Contributor's Comment: In humans, the three pathologic processes that most commonly affect the prostate are inflammation, benign nodular enlargement and neoplasia. In nonhuman primates, inflammation is more common.[7] In humans, acute bacterial prostatitis is typically caused by bacteria that cause urinary tract infections, including *Escherichia coli*, other gram-negative rods, enterococci and staphylococci. Bacteria become implanted in the prostate by intraprostatic reflux of urine from the urethra or the urinary bladder. Occasionally bacteria may spread to the prostate via



1-3. Prostate gland, cynomolgus monkey: 33% of the prostate gland is effaced by lytic necrosis. Adjacent less affected glands are compressed by the abscess and multifocally contain cellular and necrotic debris. (HE 0.63X)



1-4. Prostate gland, cynomolgus monkey: Adjacent to large areas of lytic necrosis (center), adjacent prostate glands are expanded by degenerate neutrophils and cellular debris. (HE 216X)



1-5. Prostate gland, cynomolgus: Intracytoplasmic B. pseudomallei within a macrophage. The bacterium is rod-shaped with rounded ends and is often described as having a "safety pin" appearance on electron microscopy. (Giemsa 1000X)

lymphohematogenous routes from distant sites of infection.[4] In this macaque, either route could have caused the inflammation in the prostate, but as widespread abscessation had occurred, it is more likely that the prostate was affected by hematogenous spread of *Burkholderia pseudomallei*.

Burkholderia pseudomallei is a facultative anaerobic, saprophytic, gram-negative bacterium that causes melioidosis, a zoonotic multisystemic disease. The bacterium is rod-shaped with rounded ends and is often described as having a "safety pin" appearance.[4] It can survive hostile environmental conditions including acidic environments, wide temperature ranges, nutrient deficiencies and dehydration.[3] The disease is spread through inhalation, contamination of skin wounds, or ingestion from the environment. Direct transmission from infected to naïve animals and vertical transmission are rare.[9] It is designated as a Tier 1 select agent (requiring biosafety level 3 containment) by the US Centers for Disease Control due to its natural resistance to antibiotics, potential for easy dissemination and high mortality in humans and animals.[8,9] Sporadic cases of melioidosis have been reported in Central and South America but the disease is rare in North America.[9,12] As it is endemic in Southeast Asia, northern Australia and the Indian subcontinent,[9,12] melioidosis should be a differential diagnosis for nonhuman primates imported from Asia which develop abscesses or nonspecific signs of infectious disease, regardless of the time since importation.[9] The animal presented here had been considered healthy for 1 year before presentation.

Burkholderia pseudomallei is an opportunistic pathogen, affecting many mammalian and non-mammalian species. The clinical signs and lesions of melioidosis are variable, and while septicemia is common, multisystemic suppurative or caseous inflammatory lesions are also characteristic.[9] The disease is best described in ruminants and swine where it may result in subclinical to disseminated fatal disease, depending on the route of infection, infectious dose, strain virulence, and host immune status.[12] In nonhuman primates, including rhesus, stumptail and pigtail macaques, chimpanzees and orangutans,[9,10] the most common clinical signs include anorexia, wasting, listlessness, intermittent cough, nasal discharge and mild respiratory disease that can result in bronchopneumonia, and generalized weakness. Multisystemic abscessation is common.[10] Nerve damage and necrotizing osteomyelitis have also been described.[9] In horses, donkeys and mules, a closely related bacterium, *B. mallei*, causes pyogranulomatous lymphangitis of the respiratory tract and skin,[14] commonly called glanders and farcy, respectively. It primarily occurs in Africa, Asia, the Middle East and South Africa, and human infections are often fatal if not treated.[2]

In nonhuman primates the prostate is divided into the cranial and caudal regions which are labeled according to their proximity to the urinary bladder and seminal vesicles,[7] and have distinct histological characteristics. In humans, the prostate is divided into 4 regions: the peripheral, central and transitional zones, and the anterior fibromuscular stroma.[4,6] Since proliferative lesions differ between the regions

of the human prostate, for example, carcinomas occur more frequently in the peripheral zone,[4,6] it is important to differentiate between the lobes of the prostate. In macaques, the cranial and caudal lobes of the prostate are analogous to the central and peripheral zones of the human prostate, respectively.[6,7] In addition to these anatomical similarities, some nonhuman primates (macaques, orangutans, chimpanzees, gorillas) express the prostate specific antigen (PSA), a marker of prostatic health in humans, enabling value as natural models for human prostatic disease.[5,6,7]

JPC Diagnosis: Prostate gland: Prostatitis, necrosuppurative, multifocal to coalescing, subacute, marked.

Conference Comment: *Burkholderia pseudomallei,* which has changed names numerous times, was first described in 1911 by Captain Alfred Whitmore, in a population of morphine users in Southeast Asia who developed multi-organ abscesses and septicemia. Noting its similarity to equine glanders (*Bacillus mallei*), Whitmore originally named it *Bacillus pseudomallei*, however it was also known as "Whitmore's disease" and "morphine injector's septicemia." Sporadic infections occurred in US and Japanese soldiers in World War II, and by the Vietnam War, the etiologic agent of melioidosis had been reclassified as *Pseudomonas pseudomallei*. Although less than 300 cases occurred during the conflict, many additional cases surfaced years later, which led to the nickname "the Vietnam Time Bomb."[11]

Given that *B. pseudomallei* is a zoonotic and potentially deadly disease as well as a potential bioterrorist threat, a significant amount of research is currently being conducted with regards to the pathogenesis, treatment and prevention of melioidosis. A recent study explored the role of toll-like receptors in the immune response and morbidity/mortality of the disease. Toll-like receptors are surface pattern recognition receptors expressed by various cells that recognize exogenous microbial products and signal the presence of infection to the host. The binding of pattern-associated molecular patterns (PAMPs) to TLRs initiates transmembrane signaling, generally utilizing the MyD88 protein, which leads to the activation of NF kappa β transcription factors, MAPK signaling, the expression of inflammatory cytokines and activation of the innate and adaptive immune systems.[1] TLR2 and TLR4, which bind bacterial lipoprotein and lipopolysaccharide (LPS) respectively, have previously been shown to regulate host innate immune responses in humans with *B. pseudomallei*.[13] TLR5, which binds flagellin, is defective in a small subset of people with a specific genetic defect resulting in ineffective signaling; carriers of this defect have recently been shown to have improved survival in melioidosis. This has led to the supposition that melioidosis may induce a TLR5-dependent innate immune response, with the release of IL-10, IL-8 and IL-6, among other cytokines, and that people with non-functional TLR5 have reduced sepsis and lower mortality due to an impairment of this inflammatory response.[13]

In closing, conference participants briefly discussed one possible differential diagnosis for melioidosis in a non-human primate: *Klebsiella pneumonia*, sometimes referred to as the "shipping fever of monkeys," can also cause abscess in multiple organs; however, the bacteria are usually evident microscopically.

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CASE II: DX12-72 (JPC 4017832).

Signalment: Adult female grey short-tailed opossum (*Monodelphis domestica*).

History: The animal was found dead.

Gross Pathology: The left ventricle was dilated with a cauliflower mass involving the aortic valves and the base of the aorta at the level of the branching of the coronary arteries from the aorta.

Laboratory Results: Gram stain: Gram-positive small cocci suggestive of *Streptococcus sp*.

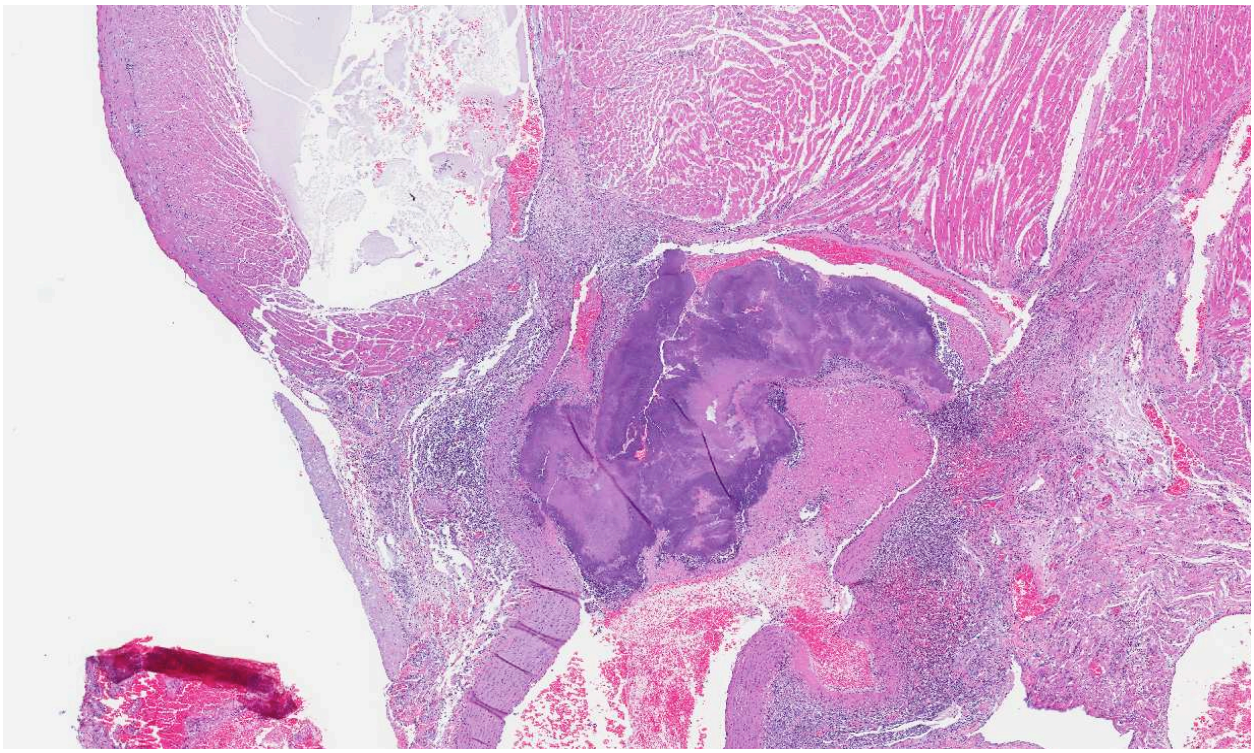
Histopathologic Description: Heart: There is a mixed inflammatory infiltrate consisting predominately of neutrophils admixed with large colonies of bacterial cocci that are attached to and destroying the aortic valves.

Contributor's Morphologic Diagnosis: Heart, aortic valve: Bacterial vegetative myocardial valvulitis.

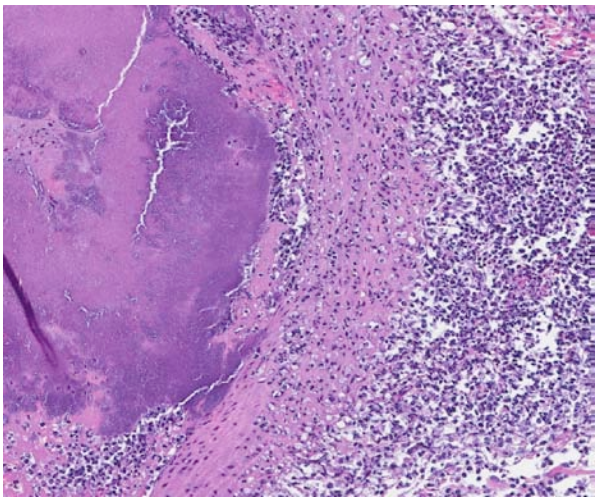
Contributor's Comment: Bacterial vegetative endocarditis is a common spontaneous occurrence in the Virginia opossum (*Didelphis virginiana*) and this marsupial has been used as an experimental animal model for *Streptococcus* bacterial endocarditis. Although cardiovascular diseases are the second most common cause of death of the laboratory grey short-tailed opossum (*Monodelphis domestica*)[1], to the contributor's knowledge, bacterial vegetative valvular endocarditis has not been previously reported in the laboratory grey short-tailed opossum.

Bacterial endocarditis primary arises from adhesion of the microorganisms to the endocardium, leading to death of the endothelium and formation and adherence of a thrombus within which large colonies of bacteria proliferate. Such proliferative growths and thrombus are called vegetative endocarditis. Although not observed in the opossum, pieces of the vegetation may break free and circulate to other organs, causing septic infarcts or abscesses. It is not uncommon for valvular endocarditis to cause cardiac dysfunction leading to congestive heart failure.

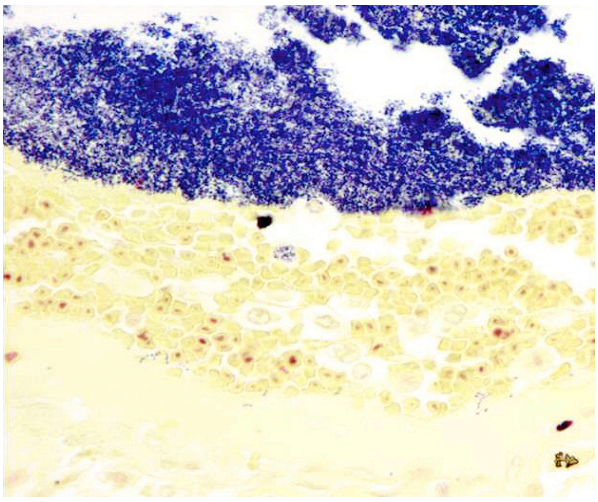
JPC Diagnosis: Heart: Valvulitis, arteritis and endocarditis, fibrinosuppurative, multifocal to coalescing, subacute, severe with fibrin thrombi, myocardial degeneration and necrosis and numerous Gram-positive bacterial cocci.



2-1. Heart, short-tailed possum: The aortic valve is effaced and the aorta lumen occluded by a septic thrombus. The inflammation extends through and beyond the wall of the aorta and dissects into the subendocardial myocardium and the base of the left atrium. (HE 10X)



2-2. Aorta, short-tailed possum. Necrosuppurative inflammation extends from the septic thrombus through the aortic wall, into the aortic adventitia. (HE 116X)



2-3. Aorta, short-tailed possum: The thrombus contains numerous gram-positive cocci. Unfortunately, the bacteria in this case were not cultured. (Brown-Brenn 1000X)

Conference Comment: In recent years, the grey short-tailed opossum has become the most commonly utilized marsupial in biomedical research, owing to its small size, docile nature, rapid growth, high fertility and relative ease of husbandry.[3] The young are not fully developed at birth, but born at a stage somewhat comparable to 40-day-old human embryos. Thus this species is often used in reproductive research.[5] *M. domestica* is also routinely used in the study of UV light-induced skin and eye neoplasia, such as melanoma. Although *M. domestica* is a hardy species with few documented parasitic or specific infectious diseases, several spontaneous pathologic conditions are reported. Most are associated with the digestive system, including rectal prolapse, which occurs primarily in females, likely related to parturition. Dermatitis, and cardiovascular disease with secondary pulmonary lesions, are also described. Pituitary adenoma is reported as the most common neoplasm in *M. domestica*, followed by uterine leiomyoma and cutaneous lipoma.[1]

Spontaneous bacterial endocarditis in the Virginia opossum (*Didelphis virginiana*) is typically due to *Streptococcus viridans* or *Staphylococcus aureus*.[4] Although culture was not performed in this case, a tissue Gram stain reveals numerous intralesional Gram-positive cocci, supporting a similar etiology in this case. In research species, bacterial endocarditis has been associated with the use of vascular access ports and intravenous catheters. Vegetative valvular endocarditis is also seen in ruminants, swine, dogs, and rarely in cats and horses. *Streptococcus* sp., *Staphylococcus* sp. and *E. coli* are frequently implicated as the etiologic agents in many species. Additionally, *Erysipelothrix rhusiopathiae* is often isolated in pigs and (occasionally) dogs, while *Bartonella* sp. is more specific to the dog or the cat. *Arcanobacterium pyogenes* is a common pathogen in cattle and *Actinobacillus equuli* can occasionally cause valvular endocarditis in horses.[2] Regardless of the inciting cause, this condition can result in valve damage and the development of congestive heart failure, or detachment of the vegetations with subsequent embolic disease.[2] In this case, because the lesion is located in the left heart at the aortic valve, the kidney would be a likely anatomic location for secondary embolic lesions.

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CASE III: PV00129C1 (JPC 4032440).

Signalment: Adult female mixed breed 4-year-old canine (*Canis lupus familiaris*).

History: This dog presented to the Center for Zoonoses Control in Natal, Brazil with anorexia, cachexia, exudative dermatitis, facial edema, and keratoconjunctivitis.

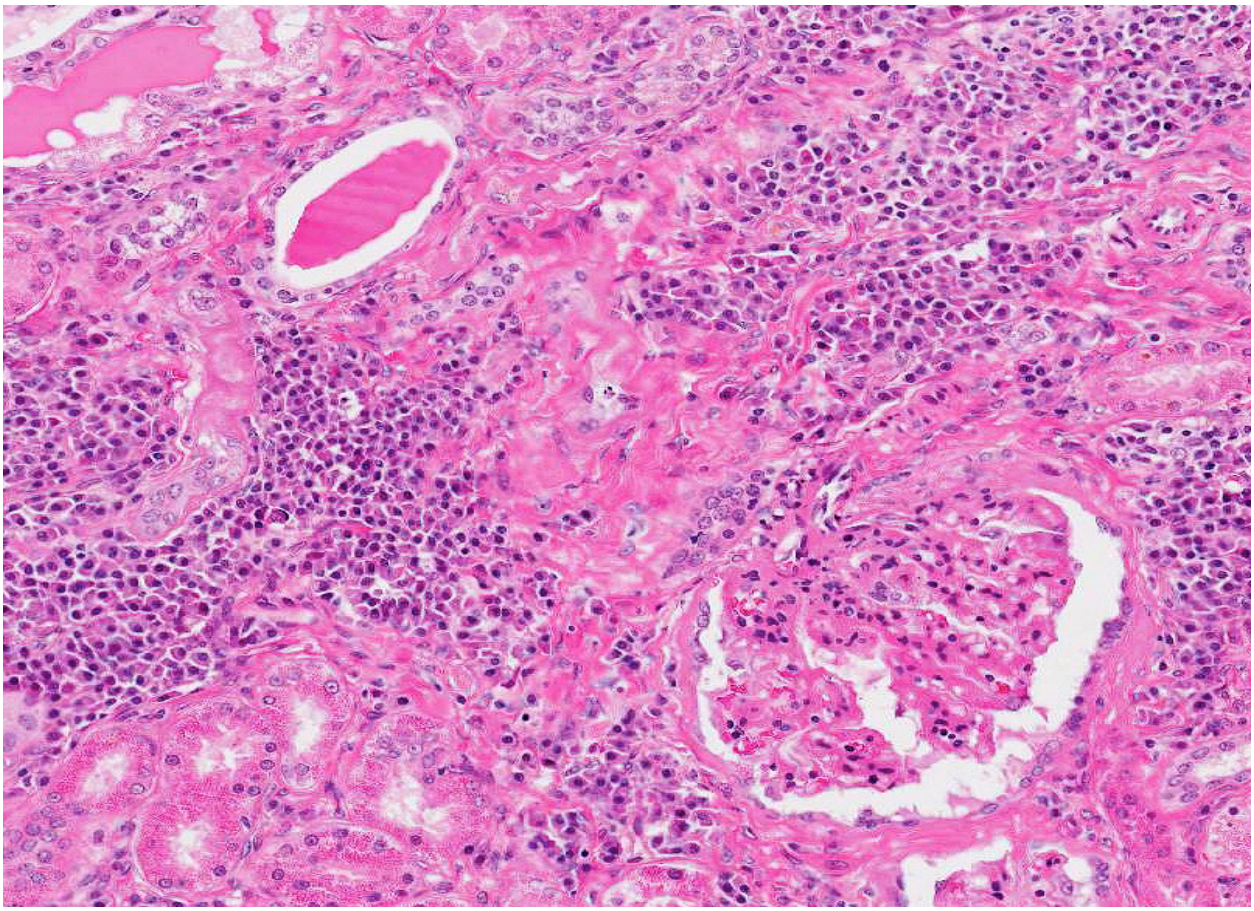
Gross Pathology: Necropsy was performed immediately after humane euthanasia. The body condition of this dog was thin with minimal subcutaneous and abdominal adipose tissue. Externally, the skin had multifocal to coalescing regions of crusting with seborrheic exudate, the muzzle of the face was markedly edematous, and the eyes had amucopurulent palpebritis and keratoconjunctivitis. There was a significant burden of ticks within the ears of the dog. The spleen was enlarged to 1.5 times normal size with a mottled irregular appearance. The liver was mildly enlarged and mottled in appearance. The kidneys were bilaterally pale and mildly enlarged with a mottled appearance and an undulating cortical surface. The bladder contained cloudy urine with numerous protein casts.

Histopathologic Description: Section of kidney with marked multifocal infiltrates of inflammatory cells including large numbers of plasma cells, macrophages, and lymphocytes. These cellular infiltrates were primarily located in perivascular and periglomerular areas of the cortical and medullary interstitium. Large foamy macrophages containing 1-3 small 1.5-3 micron amastigotes with an occasionally visible kinetoplast perpendicular to the protozoal nucleus were commonly found within areas of inflammation. Glomeruli were diffusely altered, with 10-15% being shrunken and hypocellular with increased collagen both within the glomerular tuft and Bowman's capsule (sclerosis), and others with the glomerular mesangium diffusely expanded by streaks of eosinophilic collagen with marked mesangial and endocapillary hypercellularity (membranoproliferative glomerulonephritis). Synechiae were numerous, often with the formation of glomerular crescents. Bowman's capsule was similarly expanded to 3-6 times its normal thickness by eosinophilic material in most affected nephrons. The tubular interstitium was prominently expanded by the inflammatory cell infiltrates as well as the deposition of fibrillar eosinophilic material (collagen). Distal medullary tubules were ectatic and contained hyper-eosinophilic proteinaceous concretions.

Contributor's Morphologic Diagnosis: Kidney: Glomerulonephritis, membranoproliferative, chronic, diffuse/global, severe, with prominent glomerulosclerosis and multifocal to coalescing lymphoplasmacytic and histiocytic interstitial nephritis with intra-histiocytic protozoal amastigotes; morphology consistent with *Leishmania* species *(Leishmania infantum* (synonym *chagasi))*.

Contributor's Comment: Canine leishmaniasis, primarily caused by *Leishmania infantum,* is a progressive and fatal disease with public health significance in endemic areas.[6] These endemic regions include the Mediterranean basin, northern and sub-Saharan Africa, Central and South America, and northern and northwestern China.[6] Additionally, within the United States, canine leishmaniasis is endemic with the American Foxhound breed, with sporadic occurrence in the Neapolitan Mastiff, Italian Spinone, other European origin breeds, and military service animals from the Middle East. Transmission is via one of numerous phlebotomine sandflies in endemic regions and vertical transmission independent of vector species has been documented. Dogs are also susceptible to cutaneous leishmaniasis caused by a variety of species including *L. braziliensis* and *L. panamensis.*[6]

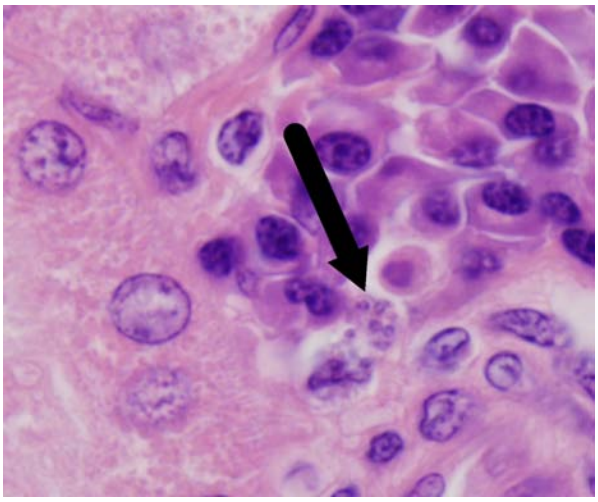
Leishmania species have a unique pathogenesis and means of persistence within host cells enabling the establishment of long-term chronic infection. After a sandfly bite, an influx of both neutrophils and macrophages occurs, even in the absence of parasites. Parasites are able to survive within neutrophils due to the ability to inhibit the acidification of the phagosome, but have not been shown to transform into amastigotes or proliferate within in the neutrophil.[9] At the time of neutrophil apoptosis, surviving parasites are phagocytosed by resident and infiltrating macrophages, where the parasites will transform into amastigotes, replicate, and establish long-term infection. Dermal dendritic cells also become infected at the site of inoculation, becoming mature and migrating to the lymph node. *Leishmania* are resistant to acidification as amastigotes, and persist in these compartments which are late endosome associated LAMP1, Rab7 positive vacuoles.[13]



3-1. Kidney, dog: The interstitium is expanded by large numbers of plasma cells and rare histiocytes. Glomeruli are enlarged and hypercellular, and tubules are often ectatic with brightly eosinophilic protein within their lumen. (HE 144X)

The immune response to all *Leishmania* species as an intracellular pathogen is dependent upon a timely and appropriate Th1 response including IL-12 production by dendritic cells and macrophages, efficient MHC II presentation, and subsequent IFNy production from T cell populations. Parasite killing is dependent primarily upon intracellular killing via superoxide and nitric oxide within phagolysosomes of infected macrophages. *Leishmania* utilize a number of immune evasion strategies to inhibit the immune response including the interruption of DC maturation, the stimulation of anti-inflammatory cytokines such as TGF-p and IL-10, the interruption of cellular signaling of the STAT pathways necessary for IFNy production, and through the induction of CD25+, FoxP3+ T regulatory cells.

Clinical presentation varies and includes dermal lesions, splenomegaly, generalized lymphadenopathy, cachexia, anorexia, muscle wasting, polyuria and polydipsia, proteinuria, keratoconjunctivitis, nail overgrowth, and hematologic abnormalities.[2,8,14] Splenic and hepatic lesions typically consist of granulomatous splenitis characterized by variable numbers of amastigote-infected macrophages, and lymphoplasmacytic and granulomatous portal and periportal hepatitis.[14] Skin lesions are one of the most common presenting signs in endemic regions and can include nonpruritic dermatitis, ulcerative dermatitis, focal or multifocal nodular dermatitis, proliferative dermatitis, or mucocutaneous ulcerative or proliferative dermatitis.[14] With these lesions, secondary bacterial pyoderma is the most common complicating co- morbidity.[8] Histologically, these lesions are granulomatous or pyogranulomatous with acanthosis, orthokeratotic and hyperkeratotic hyperkeratosis, and ulceration with serocellular crust formation.[8] Lymphoplasmacytic vasculitis and perivasculitis may also be present. Ocular lesions may also occur in approximately 16% of patients, depending on disease severity.[11] Common manifestations are conjunctivitis, blepharitis, and anterior uveitis.[11]



3-2. Kidney, dog. Histiocytes contain intracytoplasmic 2-4 µm round amastigotes with a central dark nucleus and a single rod-shaped kinetoplast. (HE 1000X)

Renal disease due to glomerulonephritis and interstitial nephritis is a common clinical sign of canine leishmaniasis due to *Leishmania infantum,* occurring in greater than 96% of symptomatic dogs. Alterations in renal function during active VL are generally reversible with *anti-Leishmania* therapy with antimonials or amphotericin B.[1,7] However, VL-associated kidney disease is progressive and without therapy can result in end stage renal disease (>25% of canine cases).[7,12] Renal lesions due to visceral leishmaniasis have been previously characterized as progressive glomerulonephritis including mesangial proliferative, membranoproliferative (MPGN), focal segmental glomerulosclerosis, and minimal change glomerulonephritis, and a smaller percentage with crescentic glomerulonephritis.[4,5] Multiple studies have evaluated the morphology and ultrastructural characteristics of renal lesions due to canine leishmaniasis.[4,5] Previous characterizations described a wide array of morphologic changes, primarily of a membranoproliferative and mesangial proliferative type.[4,5]

Dogs with symptomatic VL typically have a hypergammaglobulinemia and a high degree of circulating parasite antigen.[3] It is logical that immune complexes comprise glomerular deposits responsible for VL-associated MPGN. However, studies evaluating the proteins associated with these glomerular deposits have had conflicting results, with either presence or absence of lgG, lgM, or C3b in glomerular deposits.[5,10] All studies found a significant increase in the amount of *L. infantum* antigen and inflammatory cells within the glomerular basement membrane and mesangium.[4,5] Increased numbers of CD4+ T cells within the glomerulus of affected animals as well as increased expression of adhesion molecules ICAM-1 and P-Selectin have been characterized.[5]

JPC Diagnosis: Kidney: Glomerulonephritis, membranoproliferative, diffuse, severe, chronic, with multifocal to coalescing lymphoplasmacytic and histiocytic interstitial nephritis and intra-histiocytic protozoal amastigotes.

Conference Comment: The contributor does an excellent job of summarizing the epidemiology, pathogenesis, clinical appearance and gross/ histologic features of canine leishmaniasis. *Leishmania* sp. produces three general types of disease in veterinary medicine: cutaneous, mucocutaneous and visceral. VL has both anthroponotic (*L. donovani*) and zoonotic (*L. infantum*) forms- dogs are the primary reservoir of zoonotic leishmaniasis.[6] VL has received a significant amount of media attention in recent years, owing to hundreds of reported cases of Old World cutaneous and (less frequently) visceral leishmaniasis in US soldiers and military working dogs deployed to Iraq or Afghanistan.[15]

While there are numerous potential clinical presentations of canine leishmaniasis, diffuse mesangioproliferative or membranoproliferative glomerulonephritis and interstitial nephritis are the most common renal manifestations.[1,4] As noted by the contributor, although a type III hypersensitivity mechanism has historically been accepted as the primary mechanism of VL glomerulopathy, there is new evidence to suggest that migration of CD4+ T-cells and increased expression of adhesion molecules such as ICAM-1 and P-Selectin are also involved, while decreased apoptosis may play a role in the proliferative pattern of MPGN.[5] The basic pathogenesis of type III hypersensitivity and immune mediated glomerulonephritis involves persistent antigenemia with a slight antigen excess, which results in circulating soluble immune complexes that deposit in glomerular capillaries. These antigen-antibody complexes activate the complement cascade via the classical pathway, which induces production of C3a, C5a and C5-9 (the membrane attack complex or MAC). C5a is chemotactic for neutrophils, which release toxic proteinases, arachidonic acid metabolites and oxygen free radicals, while both C3a and C5a are potent anaphylatoxins. Additionally, the MAC is

capable of directly damaging the glomerular capillaries and mesangium.[16]

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CASE IV: G8745 (JPC 4033559).

Signalment: 3-year-old intact male ring-tailed lemur (*Lemur catta*).

History: The lemur was part of the breeding group of the German Primate Center (GPC), kept in a partly indoor and partly outdoor facility. Until found in an ill condition, the animal was an active part of his social group. He was found in the morning, apart from his family with signs of abdominal tumefaction, pressing and passive behavior. Treatment with Butylscopolamin, Metamizole and Dexamethasone followed. The general condition declined two days later, so blood samples were taken, the medical treatment was sustained and an intravenous drip was administered for rehydration.

The next day, ultrasonic examination of the abdomen was made without proper results, so a laparotomy was performed. Within the abdomen there was ascites and severe multifocal necrotizing hepatitis, so a biopsy of the liver was taken. One day after the surgery the ring-tailed lemur was found dead in his cage.

Gross Pathology: At necropsy, the lemur was in good nutritional condition. The main pathologic finding was severe hepatomegaly. The liver parenchyma was diffusely interspersed with small partly confluent whitish lesions. The spleen was enlarged and a serofibrinous peritonitis was manifest, accompanied by ascites. Parts of the small intestine, mesentery and pancreas were clotted together. In addition the mesenteric lymph nodes were hyperplastic. Focal petechial hemorrhage was found in parts of the duodenum and ileum. There was also severe, diffuse edema in the lung.

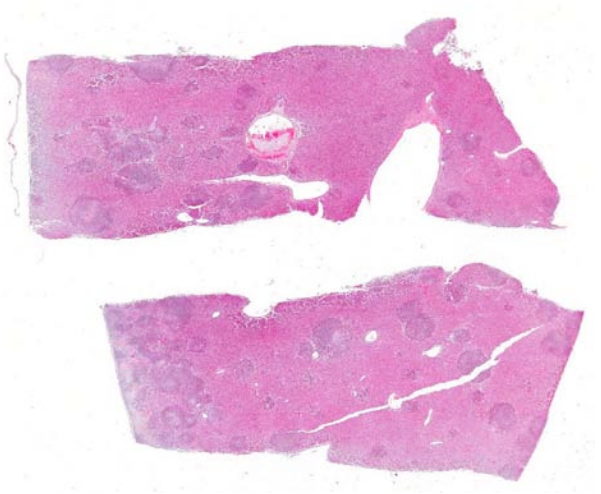
Laboratory Results: AST (392 U/L) and LDI (2079 U/L) were markedly increased. BUN (72 mg/dl) and creatinine (1.66 mg/dl) were moderately increased.

Listeria monocytogenes was isolated from heart, lung, liver, spleen, kidney and central nervous system by bacteriological culture and confirmed by PCR.

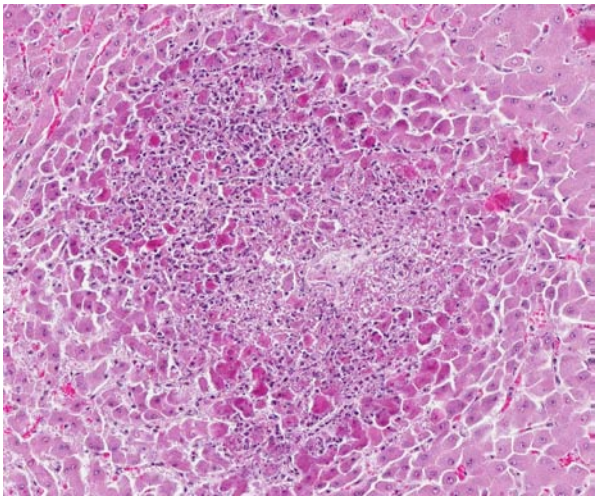
Immunohistochemistry using a polyclonal anti-*Listeria monocytogenes*-antibody revealed a positive reaction in liver, gallbladder, spleen, kidney, urinary bladder, large and small intestine, mesentery, pancreas, palatine tonsil, mesenteric lymph nodes and periorchium.

Histopathologic Description: Liver: Within the parenchyma there are numerous multifocal to coalescing areas of necrosis and inflammation, characterized by karyorrhectic debris, degradation of cells and a mild infiltration of leukocytes. The foci are sharply bounded from healthy liver tissue and randomly scattered throughout the liver.

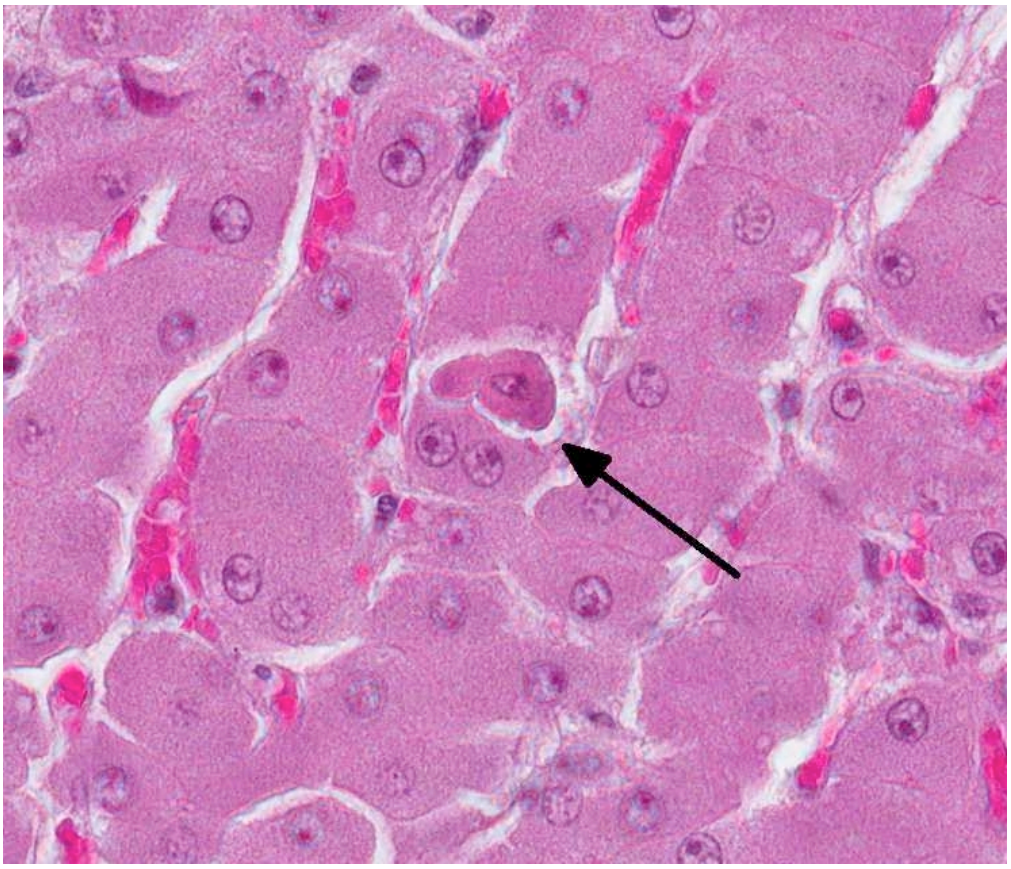
Contributor's Morphologic Diagnosis: Liver: Necrotizing hepatitis, acute, multifocal to coalescing, with necrotizing serositis, ring-tailed lemur (*Lemur catta*), nonhuman primate.



4-1. Liver, ring-tailed lemur: There are multifocal to coalescing random areas of hepatocellular necrosis that often efface portal, central veins, and vasculature. (HE 0.63X)



4-2. Liver, ring-tailed lemur: Areas of lytic necrosis are surrounded by darkly eosinophilic shrunken, degenerating hepatocytes. (HE 80X)



4-3. Liver, lemur: In less affected areas, there are individual hepatocytes that are rounded up, with darkly eosinophilic cytoplasm. (HE 400X)

Contributor's Comment: Listeriosis is an important foodborne disease, found in a large range of species. It is most frequently apparent in ruminants, but it is also an important issue in human medicine, especially in geriatrics, neonatology and diseases of immunocompromised patients.

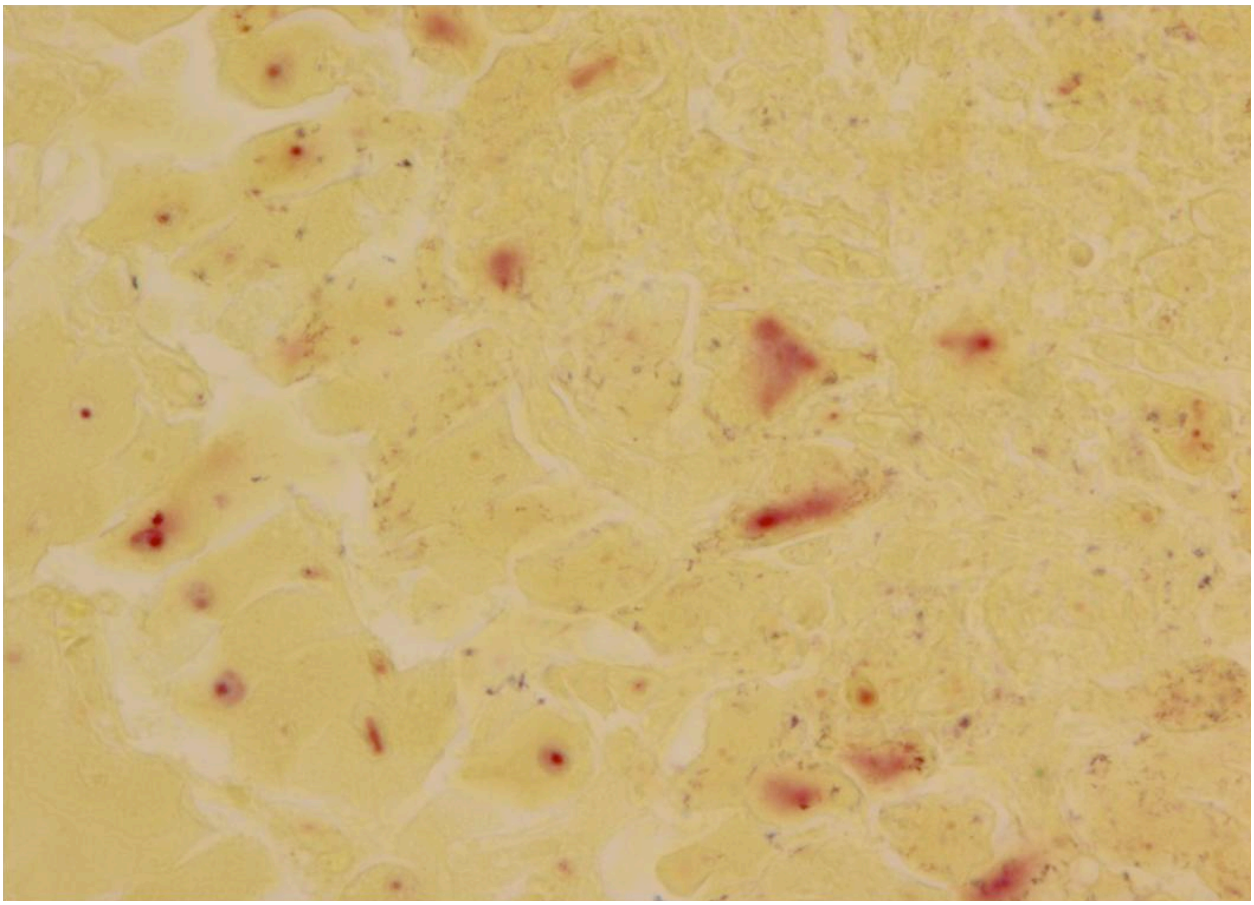
The disease is caused by *Listeria monocytogenes*, a facultative anaerobic, gram-positive, rod-shaped bacterium which has the ability to invade cells and duplicate in the cytoplasm. *Listeria monocytogenes* is a ubiquitous pathogen; it is found in the natural microbial flora in ruminants, in the feces of birds and wild animals and it persists in the environment as a saprophyte, especially on decaying vegetation, such as inadequately soured silage. *Listeria monocytogenes* is known to cause three different clinical presentations. The cerebral form, with meningitis and encephalitis, is the most common in sheep. The pregnancy-associated form leads to stillbirths, abortion and premature birth. The third form is the septicemic form. Most cases of listeriosis remain clinically silent in healthy individuals.

Relatively few cases of listeriosis are reported in nonhuman primates (NHP) and there are no case reports in ring-tailed lemurs. Most of the reported incidences in NHP are accompanied by reproductive failure with abortion, stillbirth or neonatal death due to septicemia or cerebral complications.[1,6,11] There is one report of a free-living guereza in Kenya with similar pathologic findings to this case.[8]

The severe nature and rapid progression of the disease in the present case could be secondary to immune suppression, as lymphoid depletion is noted in multiple lymph nodes throughout the body.

JPC Diagnosis: Liver: Hepatitis, necrotizing, acute, multifocal to coalescing, moderate, with hepatocellular dissociation and individual hepatocyte necrosis.

Conference Comment: *Listeria monocytogenes* is most notorious in veterinary medicine for its effects in the CNS and reproductive system, although a septicemic form appears to be the culprit in this case. In ruminants and horses, unilateral or bilateral rhombencephalitis (and occasional meningitis/meningoencephalitis), with microabscessation, is the typical clinical manifestation of listeriosis, however this bacterium can also localize to the pregnant uterus, causing sporadic abortion and stillbirth.[10] Abortion due to *Listeria* has also been reported in rabbits,[7] and listeriosis has become an increasingly significant cause of reproductive failure and fetal septicemia in non-human primates.[2] Additionally,



4-4. Liver, lemur: Few gram-positive L. monocytogenes bacilli are present at the periphery of necrotic foci. (BB 1000X)

there is at least one report of spontaneous meningoencephalitis in an immunocompetent rhesus macaque,[7] and enteric listeriosis, though infrequent, has been reported in New Zealand sheep.[4]

There is evidence that *Listeria* localizes to the ruminant brainstem via retrograde axonal migration along cranial nerve branches after crossing the oral epithelium, with potential spread into more rostral brain regions by intracerebral axonal migration. This is in contrast to human CNS infections where a hematogenous route is hypothesized.[3] It has also been suggested that E-cadherin (expressed by oral epithelium and Schwann cells) could bind internalin on the surface of *Listeria* to facilitate entry into the brainstem, however this has not yet been demonstrated.[3,4] Listerial abortions in ruminants occur during the last trimester of pregnancy with fetal infection or septicemia caused by hematogenous spread from the placenta. If the dam is infected at the beginning of the last trimester, there is rapid fetal infection and abortion with only mild maternal disease. If the infection occurs closer to parturition, dystocia with severe metritis, placentitis and septicemia are more likely.[10]

Histologic lesions of listeriosis are typically characterized by necrosis with a mild neutrophilic infiltrate.[8] Conference participants explored several potential differential diagnoses for necrotizing hepatitis in a NHP, briefly discussing *Francisella tularensis*, *Clostridium piliforme* and alphaherpesviruses. Hepatic conditions specifically reported in lemurs include cirrhosis, hemochromatosis and hepatocellular carcinoma.[5,8]

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