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<u>**CASE I**</u> – 06049-A WFUHS (AFIP 3026269).

Signalment: 12+ year-old, female, cynomolgus monkey (*Macaca fascicularis*)

History: The monkey was in quarantine after importation from Indonesia. This animal appeared to be in good health upon arrival. A tuberculin test had been performed three times during the quarantine; the first test showed a complete negative result; the second showed a questionable reaction at 48 hours observation, but then was considered negative at 72 hours observation; and the third showed marked eyelid swelling at 24 and 48 hours observation. The monkey had lost 0.2 kg of its body weight since arrival. The monkey was euthanized due to the suspicion that it had tuberculosis.

Gross Pathology: The monkey was in thin body condition and the eyelid at the tuberculin site was edematous. Numerous 1-3 mm diameter, rounded, punctate, tan, raised nodules (tubercles) were scattered on the spleen capsule and within the parenchyma. Similar tubercles were also present on the capsule and within the liver parenchyma, but were smaller in size, about 1-1.5 mm in diameter. The mesenteric lymph nodes were diffusely enlarged and prominent. No lung lesions were noted grossly.

Laboratory Results: Culture for acid fast bacteria identification is pending.

Histopathologic Description: Lung: There are multifocal, confluent granulomatous foci containing large numbers of epithelioid macrophages admixed with multinucleated giant cells, fewer lymphocytes, plasma cells, and some neutrophils with central necrosis, compressing adjacent alveoli. Adjacent peribronchial connective tissue is markedly expanded by moderate to large numbers of similar

inflammatory cells. A bronchus is segmentally eroded by the granulomas, and the remaining epithelial cells are hyperplastic and reactive (not present in some slides). The tunica intima of a medium artery is focally eroded by loose mesenchyme, interspersed with small to moderate numbers of lymphocytes and plasma cells with few small, reactive vessels (varies between slides). Some lymphatic vessels multifocally are markedly expanded by clusters of macrophages and multinucleated giant cells. The alveolar walls adjacent to the granulomas are often thickened by prominent type 2 pneumocytes. The remaining alveoli occasionally contain small to moderate numbers of alveolar macrophages or pale eosinophilic liquid material (edema). There is a focal extensive thickening of the pleura by collagenous connective tissue, and the pleura is multifocally expanded by similar granulomas.

Spleen: Replacing the parenchyma multifocally there are discrete, irregular, variably sized granulomatous foci, up to 1.5 mm in diameter. The periarteriolar lymphoid sheaths (PALS) are reduced in number and size (lymphoid depletion). Within the center of some lymphoid follicles, there are small to moderate amounts of amorphous deeply eosinophilic material (amyloid, presumed).

Granulomas were also present in liver, pancreatic lymph node, mesenteric lymph node, and tracheobronchial lymph node. Moderate numbers of nematodes (Strongylid and filarid) were present in small intestine and colon; cysts of *Sarcocystis* sp. were found in the tongue (Tissues are not submitted).

The eyelid lesions demonstrated that the connective tissue and muscle within the dermis were multifocally dissociated, fragmented, and expanded by fibrinous material (edema and inflammation) and large aggregates of lymphocytes, plasma cells, macrophages and fewer neutrophils, particularly surrounding blood vessels (perivascular), severe in a focally extensive area (Type IV hypersensitivity reaction, tissue is not submitted).

Acid fast staining was performed on the lung, spleen, and liver. There were myriads of acid fast bacteria intercellular and intracellular of phagocytic cells within the focus of necrosis in the lung, but acid fast bacteria were rarely present in the spleen and liver.

Contributor's Morphologic Diagnoses:
1. Bronchopneumonia, necrotizing, granulomatous, severe, focally extensive, with multifocal granulomatous lymphatic emboli and myriad intralesional acid fast bacteria, consistent with *Mycobacterium tuberculosis*.
2. Splenitis, necrotizing, granulomatous, moderate to severe, multifocal with mild amyloidosis and lymphoid depletion.

Contributor's Comment: *Mycobacterium tuberculosis* is the principal cause of tuberculosis in humans and nonhuman primates. It is occasionally encountered as a cause of tuberculosis in dogs, but cattle and cats are relatively resistant. Guinea pigs and hamsters are highly susceptible, but rabbits and birds are resistant.[1]

M. tuberculosis is an obligatory aerobic, intracellular pathogen, which has a predilection for the lung tissue. The tubercle bacilli enter the body via the respiratory route; the bacilli may massively disseminate lymphohaematogenously (miliary tuberculosis) from a pulmonary or extrapulmonary focus via embolisation to the vascular beds of various organs. Organs with high blood flow, e.g. spleen, liver, lungs, bone marrow, kidneys, and adrenals are frequently affected; regional lymph nodes are commonly affected through the lymphatic system.[2] The granulomas may also rupture into airways, allowing the mycobacteria to be released and transmitted to other hosts via aerosols.

The early event following inhalation of *M. tuberculosis* is engulfment by alveolar macrophages (phagocytosis). It is well known that mycobacteria have the ability to survive in phagosomes by inhibiting phagolysosomal fusion. Early studies showed that sulphatides (cell wall component), derivatives of multinucleated trehalose-2 sulfate, a lysosomotropic polyanionic glycolipid, have a major role in the inhibition of phagolysosomal fusion. More recent studies proposed several mechanisms for the fusion inhibition, including high ammonia production by the mycobacteria, glycosylated phosphatidylinositol lipoarabinomannan (ManLAM) lipid rich cell component, and mycobacterial protein kinase G.[3, 4, 5]

Within 2-6 weeks cell mediated immunity (CMI) develops, and there is an influx of lymphocytes and activated macrophages into the lesion resulting in granuloma formation. The exponential growth of the bacilli is checked and dead macrophages form a region of caseous necrosis. The bacilli are contained in the caseous centers of the granulomas.[2] The bacilli may remain indefinitely within the granuloma, become re-activated later or may get discharged into the airways after erosion of airways. If the bacteria in the lesion are eventually overcome, the tubercle is reduced to a small mass of fibrous and hyaline scar tissue. When miliary tuberculosis occurs in human beings, the lesions appear alike and are termed "soft" or "exudative" and the lesions often reveal acid fast bacilli. Acid fast bacilli are less abundant in more chronic "hard" tubercles.[6]

Nonhuman primates (NHP) acquire classic tuberculosis infection by contact with other nonhuman primates or humans through inhalation or the digestive route. These infected animals can become reservoirs, causing outbreak of disease. Clinical diagnosis of tuberculosis in NHP can be difficult because infected monkeys may only show mild behavioral changes like anorexia and lethargy. Occasionally, infected monkeys may suddenly die while appearing in good body condition.

Tuberculosis in cynomolgus monkeys has many comparable gross and histopathology changes consistent with different stages in human infection; which makes the cynomolgus monkey an animal model for human tuberculosis.[7]

Detection of tuberculosis in the NHP has relied on tuberculin skin response, serological testing, histopathology, microscopy and culture identification. Among these, the most frequently used methods are culture identification and the tuberculin skin test (also known as Purified Protein Derivative), the latter being a routine test in quarantine and preventive medicine protocols. However, the PPD test is not adequately sensitive or specific in many species and the rate of false negatives is high.

Tuberculin is the protein lipopolysaccharide component of *M. tuberculosis* that is commonly used in diagnosis of subclinical tuberculosis infection. The edema at the site of tuberculin injection is the result of a type IV hypersensitivity (cell mediated or delayed type hypersensitivity) reaction, that usually appears in 8-12 hours, and reaches a peak in 24-72 hours. On intradermal injection of tuberculin in an animal previously exposed to the mycobacteria, the memory Th1 cells interact with the antigen on the surface of antigen-presenting cells and are activated (undergo blast transformation and proliferation). These changes are accompanied by the secretion of the Th1 type cytokines (IL-12, IFN-γ, IL-2, TNF-α) which attract mononuclear cells (T cells and macrophages) to the area.[8]

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AFIP Diagnoses: 1. Lung: Granulomas, multifocal to coalescing, severe, cynomolgus monkey (*Macaca fascicularis*), nonhuman primate.
2. Spleen: Splenitis, granulomatous, multifocal to coalescing, moderate.
3. Spleen: Lymphoid depletion, diffuse, mild.
4. Pancreas: No significant lesions.

Conference Comment: The contributor provides an excellent and thorough overview of *Mycobacterium tuberculosis* to include species susceptibility and pathogenesis. Four species of *Mycobacterium* are considered causes of "classic" tuberculosis – *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti* (*M. tuberculosis* complex). Though there is some species predilection with each, all three can infect a wide range of species, and especially immunocompromised animals. The most common agents of tuberculosis in primates are *Mycobacterium tuberculosis* and *M. bovis*. Organisms in the *M. avium-intracellulare* group are frequently isolated in macaques with mycobacterial infections in which tubercle formation is not a feature. *M. avium-intracellulare* infections in nonhuman primates resemble Johne's disease primarily affecting the intestinal mucosa and mesenteric lymph nodes.[1,9,10]

There is slide variability with some slides containing sections of pleura attached to myocardium.

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CASE II – G 7282 (AFIP 3034591).

Signalment: 5-year-old, intact female, rhesus macaque (*Macaca mulatta*), non-human primate.

History: This monkey was inoculated with simian immunodeficiency virus (SIV$_{mac}$) on 10/02/04 via the tonsillar route. Eighty-five weeks after the tonsillar challenge

the animal showed a deteriorating general condition and reduced appetite. The monkey was euthanized on 26/11/05 due to a poor prognosis.

Gross Pathology: At necropsy the rhesus macaque was in moderate nutritional condition. Severe ascites with amber fluid was observed in the abdominal cavity. The liver showed moderate hepatomegaly with blunt margins and multiple hemorrhagic foci throughout the parenchyma. Further findings included moderate hypertrophy of the left ventricle, generalized hyperplasia of the lymph nodes and severe follicular hyperplasia of the spleen.

Histopathologic Description: At microscopic examination the liver parenchyma reveals multiple coalescing foci of hepatocellular necrosis with multifocal hemorrhages and a sparse inflammatory reaction, characterized by few neutrophils in areas of necrosis and a mild periportal mononuclear cell infiltrate. Numerous intranuclear basophilic round to oval shaped inclusion bodies are present within the hepatocytes.

Immunohistochemical staining for SV40 performed on sections of formalin-fixed liver was negative.

Transmission electron microscopy of liver samples revealed adenovirus like particles in the nucleus of hepatocytes.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, necrotizing, acute, multifocal to coalescing, severe, with intranuclear basophilic inclusion bodies, rhesus macaque (*Macaca mulatta*), non-human primate.

Contributor's Comment: The family *Adenoviridae* is subdivided into four genera: *Mastadenovirus* affecting mammals, *Aviadenovirus* which infect birds, *Atadenovirus* with a broad host range from several vertebrate classes including reptiles, birds and mammals and *Siadenovirus* which has been isolated from amphibians (frog) and birds (turkey pheasant, chicken). Adenoviruses are non enveloped, double-stranded DNA viruses that consist of a capsid, fibers, a core, and associated proteins. The icosahedral capsid has a diameter of 80-110 nm. Viral replication is located within the nucleus of host cells and results in characteristic inclusion bodies visible by light microscopy.[1,2]

The recognized diseases associated with adenovirus infection in humans predominantly involve the respiratory tract (pneumonia), the GI tract (gastroenteritis), the eye (keratoconjunctivitis) and the genitourinary tract (cystitis, urethritis, cervicitis). Virus may be introduced through contact, respiratory droplets or ingestion. Immunocompromised patients are especially susceptible to

adenovirus infections and opportunistic adenovirus induced disease with high case-fatality rates is a frequent finding in organ transplant recipients and AIDS patients.[3]

Experimentally, several adenoviruses have been shown to cause malignant neoplasms in newborn hamsters and other laboratory animals and to transform cells in tissue culture. However, a causal relationship to spontaneous neoplasms has not been established yet.[1]

At least 27 serotypes of adenoviruses have been isolated from nonhuman primate species, including macaques (*Macaca* spp.), African green monkeys (*Cercopithecus aethiops*), baboons (*Pan* spp.), squirrel monkeys (*Saimiri sciureus*) and cotton top tamarins (*Saguinus oedipus*). However in immunocompetent animals, infection with adenovirus most often leads to subclinical disease and reports of adenoviral pneumonia, gastroenteritis and conjuctivitis are few.[4]

In immunocompromised monkeys simian adenovirus is an uncommon, but significant potential opportunistic pathogen and has been associated with segmental enteritis involving the ileum and necrotizing pancreatitis in SIV infected rhesus macaques.[4,5,6] Necrotizing hepatitis is an extremely rare manifestation of adenovirus infection in nonhuman primates. Individual cases have been described in rhesus monkeys (*Macaca mulatta*, African green monkeys (*Cercopithecus aethiops*) and chimpanzees (*Pan troglodytes*) that all showed evidence of immunosuppression.[7]

In the present case etiological diagnosis of adenoviral hepatitis was based on transmission electron microscopy with evidence of paracrystalline arrays of adenovirus in the nucleus of hepatocytes (Fig. 1).

Other causes of necrotizing hepatitis in rhesus monkeys include hepatitis A and hepatitis B, SV40 and herpesvirus infection. In domestic animals adenoviruses have been recovered from cattle, sheep, pigs, horses, mice and dogs and are most often associated with pneumonia and enteritis. However, with the exception of infectious canine hepatitis, most are not serious causes of disease in animals other than those that are immunocompromised.[1]

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AFIP Diagnoses: 1. Liver: Hepatitis, necrotizing, multifocal, random, marked, with fibrin, hemorrhage, edema, and eosinophilic to basophilic intranuclear inclusion bodies, rhesus macaque (*Macaca mulatta*), nonhuman primate.
2. Liver: Hepatitis, portal, lymphocytic, multifocal, mild.
3. Gallbladder, lamina propria: Edema, diffuse, marked.

Conference Comment: The contributor provides a thorough overview of adenoviruses. As mentioned by the contributor, adenoviruses are currently classified into four genera. Below is a comparative list of adenoviruses and some of the diseases they cause:[1,2,4,8,9,10]

Genus Aviadenovirus (Group 1 avian adenoviruses)**:**
- Fowl, goose, duck, pigeon, turkey adenovirus: Inclusion Body Hepatitis (IBH); hydropericardium syndrome; respiratory disease; necrotizing pancreatitis and gizzard erosions
- Quail Bronchitis (avian adenovirus Type 1)

Genus Siadenovirus:
- Marble spleen disease (MSD) (Adenovirus Type 2): Pheasants; splenic necrosis, respiratory edema, congestion and asphyxia
- Hemorrhagic enteritis (HE) (Adenovirus Type 2): Young turkeys; bloody droppings, death
- Avian adenovirus splenomegaly virus (AASV)
- Frog Adenovirus

Genus Atadenovirus:
- Egg drop syndrome (subgroup 3 avian adenovirus): Laying hens, viral replication in pouch shell gland epithelium; intranuclear inclusion bodies
- Ovine, bovine, duck, possum adenoviruses
- Adenoviral Hemorrhagic Disease in California mule deer and black-tailed deer: Vasculitis with endothelial intranuclear inclusion bodies, pulmonary edema, hemorrhagic enteropathy; produces similar lesions to Bluetongue virus and Epizootic Hemorrhagic Disease (EHD) (orbiviruses)
- Reptilian Adenoviruses
  - Bearded dragon, snake, chameleon, gecko

Genus Mastadenovirus:
- Human adenovirus: Respiratory disease, enteritis; keratoconjunctivitis
- Simian adenovirus (27 different viruses): Mostly subclinical; some secondary to immunosuppression, mild to moderately severe respiratory and enteric disease, keratitis/conjunctivitis
- Canine adenovirus 1: Infectious canine hepatitis
- Canine adenovirus 2: Necrotizing bronchiolitis and alveolar epithelialization; most cases secondary to immunosuppression
- Equine adenovirus: Mild respiratory disease except in CID Arabian foals where adenoviral infection leads to severe bronchiolitis, atelectasis, and pancreatitis
- Bovine adenovirus: Respiratory tract disease, pyrexia, KCS, colic, associated with respiratory and enteric disease in calves but not considered the primary

pathogen in either syndrome
- Ovine adenovirus: Respiratory tract disease, conjunctivitis, enteritis
- Porcine adenovirus: Widespread, mostly subclinical, pneumonia, enteritis associated with encephalitis and diarrhea
- Murine adenovirus: Oncogenic in newborns, experimentally induce CNS lesions
- Guinea Pig Adenovirus: Pneumonitis
- Adenovirus can experimentally cause tumors in hamsters and rats
- Wildlife: Brown bear, coyotes, foxes, wolves, skunks and raccoons are also susceptible to CAV-1.

Note: Several viral isolates share the same name with isolates from other genera and are only differentiated by letter designators. For a complete table of the adenoviruses, readers are encouraged to visit the ICTV website listed in reference 8.

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CASE III – S0511870-1 (AFIP 3044695).

Signalment: 1-year-old, female, black face, *Ovis aries*, sheep.

History: CNS disease of 2 days duration, affecting approximately 1% of the flock.

Gross Pathology: Bilateral and focal symmetrical areas of malacia in basal ganglia, thalamus and cerebellar peduncles

Laboratory Results: *Clostridium perfringens* type D isolated from small intestine. *Clostridium perfringens* alpha and epsilon toxin detected in small intestinal contents by capture ELISA.

Histopathologic Description: Corpus striatum: At the subgross level there are several foci of pallor (rarefaction) with loss of tissue architecture (degeneration) within the ventral aspect of the internal capsule (the internal capsule is the more acidophilic white matter track that crosses the section). In these areas there is multifocal perivascular proteinaceous edema surrounding veins and a few arterioles. The endothelium of these vessels shows hypertrophy and there is an increased number of neutrophils within the lumen and a few of these cells are occasionally seen in the perivascular space together with a few lymphocytes. Diffusely in the areas of rarefaction, there is vacuolation of the neuropil (spongiosis) and dilated axon sheaths with or without swollen axons (spheroids). There is also gliosis, mostly represented by large and vesicular astrocytes, some of which have a moderate amount of acidophilic cytoplasm (gemistocytes). Gitter cells are not a prominent feature in this section. The gray matter surrounding the internal capsule (basal ganglion) looks mostly unaffected.

Contributor's Morphologic Diagnosis: Corpus striatum (internal capsule): Degeneration of white matter, focally extensive, bilateral with proteinaceous perivascular edema

Contributor's Comment: *C. perfringens* type D produces a peracute, acute or chronic neurological condition in sheep, characterized by sudden death or neurological signs including blindness, opisthotonos, convulsions, bleating and

recumbency. Diarrhea is occasionally observed, although this is not a common clinical sign in sheep.[4] Small intestinal changes, if present, consist of hyperemic intestinal mucosa with slightly-to-markedly red fluid contents. Colitis may occur, but is not a consistent finding in sheep enterotoxemia. Several other gross findings, such as excess pericardial pleural and/or abdominal fluids with or without fibrin strands, serosal petechiation, and lung edema, suggest, but are not specific for, type D enterotoxemia. Pathognomonic gross changes in sheep are in the brain, and consist of herniation of the cerebellar vermis (cerebellar coning) and/or focal symmetrical encephalomalacia (FSE). While cerebellar coning can be found in peracute and acute cases, FSE is seen only in chronic cases and is characterized by dark, hemorrhagic foci in corpus striatum, thalamus, midbrain, and cerebellar white matter cores.[1,2,3]

Microscopic changes in the brain of sheep are unique and pathognomonic, although they are not present in all cases.[2,5,6] The most consistent change is perivascular proteinaceous edema (microangiopathy), consisting of acidophilic accumulations of protein surrounding small and medium sized arteries and veins.[2] This lesion is very prominent in the sections of the submitted case. Perivascular edema can already be seen a few hours after onset of clinical signs. To the authors' knowledge, there are no other conditions of sheep that produce this highly proteinaceous perivascular edema of the brain in sheep and this change is therefore diagnostic for type D enterotoxemia. In chronic disease, a lesion characterized by degeneration and/or necrosis of white matter can be observed.[3] This lesion is the histological counterpart of the gross change of FSE and it is characterized by degeneration of white matter, hemorrhage, and astrocyte and axonal swelling as seen in the accompanying section. Perivascular edema and degeneration and necrosis of brain parenchyma are always bilateral and symmetrical and they have been described most frequently in corpus striatum, internal capsule, thalamus, mid brain, cerebellar peduncles, and cerebellar white matter cores.[1,2] These areas are not exclusively affected, and lesions can sometimes be seen in other parts of brain, such as cortex and hippocampus.[2] Because histological lesions are observed in most, but not all cases of ovine enterotoxemia, these changes are a very useful indicator of enterotoxemia, but absence of these lesion does not therefore preclude a diagnosis of this disease in sheep. The most widely-accepted criterion in establishing a definitive diagnosis of type D enterotoxemia in both sheep and goats is detection of epsilon toxin in intestinal contents.[6] The currently submitted case tested positive for epsilon toxin in intestinal content thus confirming a diagnosis of enterotoxemia.

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AFIP Diagnosis: Brain, white matter: Necrosis and loss, multifocal, with edema, sheep (*Ovis aries*), ovine.

Conference Comment: The contributor provides an excellent summary of focal symmetric encephalomalacia in sheep caused by *Clostridium perfringens* type D to include clinical findings and pathognomonic gross and light microscopic findings.

Clostridium perfringens is an anaerobic, spore-forming, Gram-positive bacillus that causes disease through the elaboration of toxins within the gastrointestinal tract. High starch diets facilitate clostridial overgrowth. *C. perfringens* type D produces alpha and epsilon toxin. Epsilon toxin is secreted as an inactive prototoxin in the gut, with activation through cleavage by trypsin. Epsilon toxin binds endothelial cell surface receptors resulting in opening of tight junctions, disturbed transport processes, and increased vascular permeability resulting in vasogenic edema, swelling of astrocytic foot processes, hypoxia, ischemia, and necrosis. Additionally, some of the effects of epsilon can be mediated by the adenyl cyclase/cAMP system.[7,8,10]

Enterotoxemia caused by *C. perfringens* type D is a common disease of sheep and goats, and has been reported in calves. In sheep, the disease most frequently occurs in fattening lambs and is known as "overeating disease", or as "pulpy kidney disease" (due to the rapid postmortem autolysis of the kidneys in some animals dying from the condition). In sheep, the disease is predominately characterized by central nervous system (CNS) signs and lesions, as was observed in this case. In the gastrointestinal tract, there is peritoneal hemorrhage, mucosal congestion of the intestines, superficial desquamation of the intestinal epithelium, and numerous bacilli in the intestinal contents. In goats, the disease primarily occurs in the gastrointestinal tract, and CNS signs and lesions occur less frequently than in sheep. Additionally, lesions in the gastrointestinal tract of goats more commonly affect the colon, rather than the small intestine.[4,7,8,9]

There was some variation between slides with neutrophils present in some sections.

Contributor: California Animal Health and Food Safety Laboratory, San Bernardino Branch, School of Veterinary Medicine, UC Davis, http:/cahfs.ucdavis.edu

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CASE IV – 06-0111 (AFIP 3028791).

Signalment: Adult male vasectomized common marmoset (*Callithrix jacchus*) that was pair housed with a female marmoset.

History: The marmoset had its tail bitten by another marmoset several weeks ago followed by surgical amputation. Started on IM enrofloxacin on 21 March 06. On the morning of 22 March 06, animal presented as weak with opisthotonos & hypothermia & was given SQ saline & IV cefazolin. The monkey developed seizures & was subsequently euthanized. Septic shock was suspected.

Gross Pathology: There is adequate body fat & normal hydration status. Both inguinal lymph nodes are enlarged & SQ edema of left inner thigh. The tail is amputated 1/3 from tip & over 1/2 of the remaining tail is red & black. There are sutures at the amputation site. The liver is diffusely pale with an accentuated lobular pattern. Lungs do not collapse & are congested. All other organs – NSGL.

Histopathologic Description: This is a section of tail. There is diffuse to multifocal, depending on the section, epidermal and superficial dermal coagulative necrosis with numerous neutrophils and abundant large colonies of coccobacilli. In some sections, the necrosis also involves the panniculus. There is multifocal edema and

often vessels are partially or completely blocked by fibrin thrombi. The neutrophils often extend into the panniculus and underlying skeletal muscle bundles. In some sections, there is scattered myocyte degeneration and necrosis. With the Brown and Hopps staining method (not included), the coccobacilli are Gram-negative.

Contributor's Morphologic Diagnosis: Tail: Necrosis, epidermal and dermal, with edema, fibrin thrombi, moderate suppurative dermatitis, panniculitis, and myositis, and numerous coccobacilli.

Other histopathologic findings included diffuse pulmonary interstitial edema and congestion, diffuse thymic involution, and moderate hepatic hemosiderosis and lipidosis.

Contributor's Comment: The history, gross findings, and histopathologic findings are all consistent with bacterial sepsis as the cause of this marmoset's morbundity. Sepsis may be caused by infection with Gram-negative bacteria and subsequent endotoxin production (lipopolysaccharide or LPS), Gram-positive bacteria, fungi, or viruses.[1] In this case, the most likely cause is Gram-negative bacterial endotoxin.

Sepsis is a generalized inflammatory response that results from triggering of the body's defense mechanisms (cytokine release; neutrophils, monocyte, and endothelial activation; neuroendocrine reflexes; and complement, coagulation, and fibrinolytic system activation) by microbial invasion, extensive tissue injury, or ischemia/reperfusion injury.[1]

The pathogenesis of Gram-negative sepsis involves the innate immune system. Local phagocytic cells (primarily macrophages and neutrophils) recognize microbes via several types of membrane receptors, including different types of Toll-like receptors (TLRs). Specifically, TLR-4 recognizes Gram-negative bacterial LPS. The "lipid A" portion of LPS is recognized by and bound to a circulating acute-phase protein, LPS-binding protein (LPB), which greatly enhances the binding of LPS to the CD14 receptor on the phagocytic cells. Once LPS is bound to CD14, LPB dissociates and the LPS-CD14 complex physically associates with TLR-4 and an extracellular accessory protein, MD2. Ligand binding causes recruitment of cytoplasmic signaling molecules MyD88 (an adaptor protein) and IL-1 Receptor Associated Kinase (IRAK) into the complex. IRAK undergoes autophosphorylation, dissociates from MyD88, and subsequently activates TNF-Receptor Associated Factor-6 (TRAF-6). TRAF-6 then activates the I-κB Kinase cascade, leading to NF-κB transcription factor activation. In some cell types, the MAP Kinase cascade is also activated, leading to AP-1 transcription factor activation. This signal transduction and transcription factor up-regulation results in leukocyte activation, cytokine/mediator synthesis and secretion, and general immune stimulation.[2,3]

The effects of LPS and the secondarily induced effector molecules (especially TNF, IL-1, IL-6, IL-8, IL-12, NO, and PAF) varies depending on the level of LPS and the number of phagocytes activated. At low doses, LPS activates local phagocytes, endothelial cells, and complement to enhance the local acute inflammatory response and improve infection clearance. At moderate levels, more systemic effects occur (fever, hepatic acute-phase protein production), in addition to the local vascular effects. With high levels, septic shock develops, resulting in systemic vasodilation, decreased cardiac output, widespread endothelial injury and activation, and disseminated intravascular coagulopathy (DIC).[4]

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AFIP Diagnosis: Tail, transverse section: Dermatitis and cellulitis, neutrophilic and necrotizing, diffuse, moderate, with fibrin, edema, hemorrhage, thrombosis, and myriad intra-epidermal colonies of bacilli, common marmoset (*Callithrix jacchus*), nonhuman primate.

Conference Comment: The contributor provides a concise and thorough overview of the pathogenesis of Gram-negative sepsis.

Gram-positive bacteria can trigger sepsis and septic shock by producing and releasing exotoxins that act as "superantigens" and by the release of cell membrane fragments (e.g. peptidoglycans, teichoic acids, and lipoteichoic acids). Superantigens are polyclonal T-lymphocyte activators that induce systemic inflammatory cytokine cascades similar to those occurring downstream in septic shock. Superantigens bind to class II MHC molecules and V_β domains of the T-lymphocyte antigen receptor (TCR). This binding occurs outside of the normal antigen binding site and activates all T lymphocytes expressing the same V_β domains irrespective of their antigen specificity resulting in the activation of numerous T lymphocytes and the elaboration of cytokines. Superantigens are subclassified as either exogenous or endogenous. Exogenous superantigens are produced by bacteria and include some enterotoxins, toxic shock syndrome toxin (TSST1), and exfoliating toxin. Endogenous superantigens are specific cell-membrane molecules produced during viral infections.[1,4,5]

Contributor: USAMRICD, Comparative Pathology Branch,
http://usamricd.apgea.army.mil/

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