Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2013-2014

Conference 20

26 March 2014

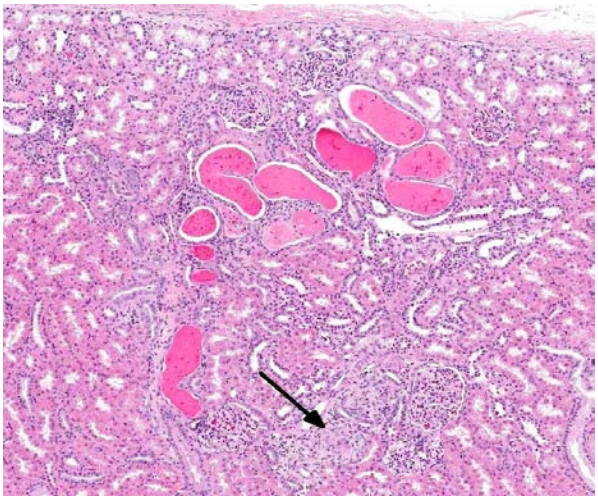
CASE I: Case 1 (JPC 3167249).

Signalment: Mature adult male cynomolgus macaque, (*Macaca fascicularis*).

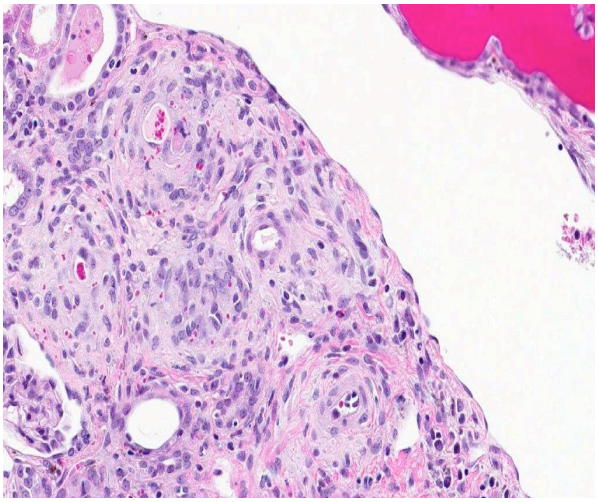
History: This animal was treated with Cyclosporine A for 30-days before being euthanized and necropsied due to decreased activity, food consumption, and body weight.

Gross Pathology: Both kidneys were brown and the heart contained a small focus of epi/ myocardial hemorrhage.

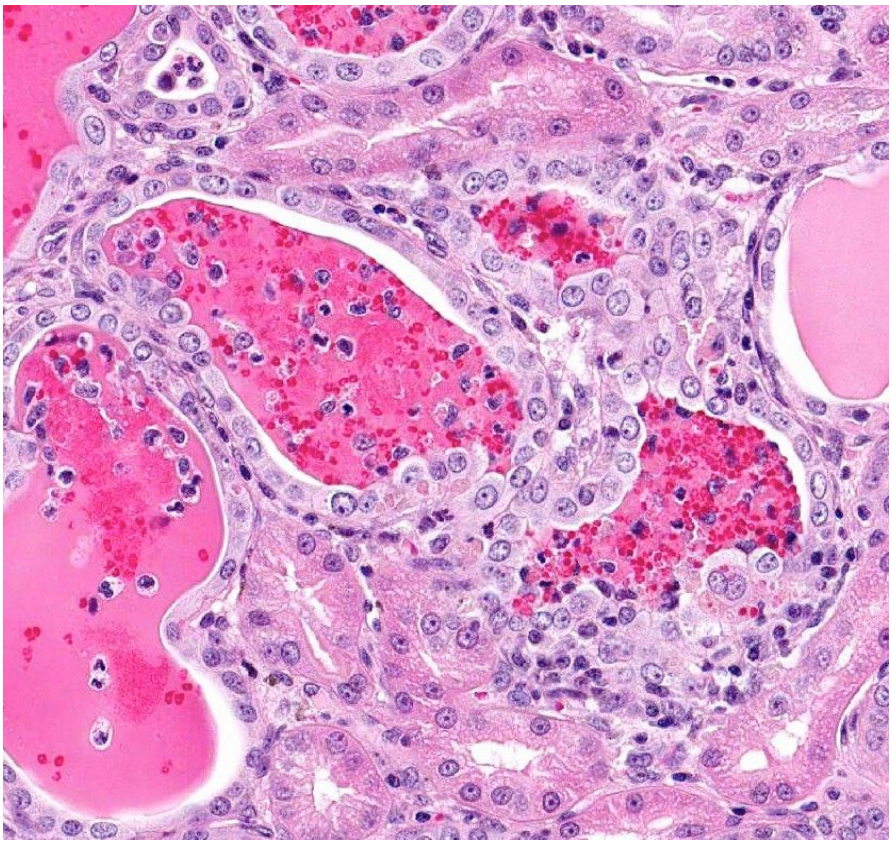
Laboratory Results: Only hematology parameters were evaluated. There was a regenerative macrocytic microchromic anemia, and schistocytes were noted upon microscopic examination of the blood smear.



1-1. Kidney, cynomolgus macaque: Segments of the kidney are hypercellular with enlarged glomeruli, ectatic tubules with protein casts, and markedly enlarged arterioles (arrow). (HE 60X)



1-2. Kidney, cynomolgus macaque: Renal arterioles are tortuous and markedly expanded by disorganized hyperplastic smooth muscle cells, extracellular protein and extruded erythrocytes. (HE 250X)



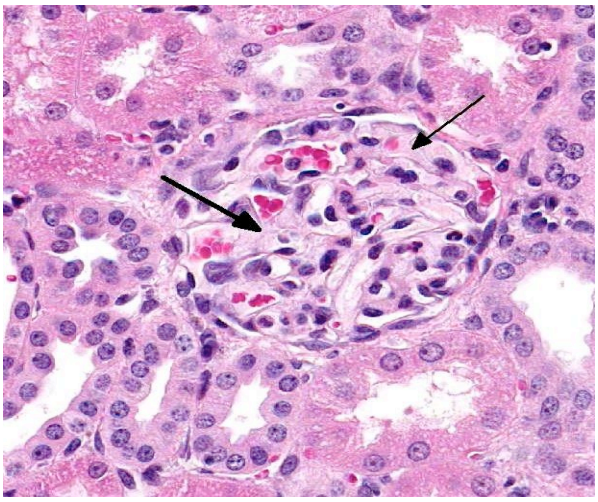
1-3. Kidney, cynomolgus macaque: Multifocally, tubules are ectatic, contain protein and cellular casts, and are lined by basophilic cuboidal epithelial cells with large nuclei (regenerative tubules). (HE 360X)

Histopathologic Description: There is minor variation between slides, but all slides contain the following main histopathologic features: Numerous arterioles in the interstitium of the cortex and corticomedullary junction have marked mural expansion by amphophilic myxomatous material and hypertrophy of medial smooth muscle cells, which are rarely necrotic, and less often contain infiltrating fibroblasts and extravasated red blood cells. Affected vessels have lumina which are reduced in diameter to completely occluded, are sometimes lined by hypertrophied endothelial cells, and rarely contain fibrin. Other vasculature sometimes contains circulating erythroid precursor cells. Cortical tubules adjacent to affected vessels are occasionally effaced, but more often are lined by epithelial cells with large vesicular nuclei and lightly basophilic cytoplasm (regenerative cells). Cortical tubules are sometimes dilated and contain either protein casts, casts comprised of fragmented red blood cells, and/or a small amount of necrotic debris. Scattered medullary tubules also contain protein casts. Scattered glomeruli are mildly hypercellular with thickened capillary basement membranes, and contain occasional adhesions (synechiae) to Bowman's capsule and/or hypertrophy of parietal cells. There are a few foci in which the cortical interstitium is minimally expanded by fibrous connective tissue and small numbers of lymphocytes and plasma cells.

Contributor's Morphologic Diagnosis:
Kidney:
1. Marked multifocal arteriopathy
2. Moderate multifocal subacute renal cortical tubular degeneration and regeneration with tubular dilation and casts
3. Mild multifocal membranoproliferative glomerulopathy
4. Minimal multifocal chronic interstitial nephritis

Contributor's Comment: The renal lesions[3,4,5,7] and hemolytic anemia[2,3] are consistent with cyclosporine or other calcineurin inhibitor (tacrolimus) toxicity in primates. Most of the literature pertaining to calcineurin inhibitor toxicity refers to humans, but there are reports of such in tacrolimus treated rhesus monkeys[3] and streptozotocin-diabetic cyclosporine treated cynomolgus monkeys.[7]

A recent review paper on calcineurin inhibitor nephrotoxicity in humans describes the chronic morphologic features as nodular hyaline material deposition in the tunica media of afferent arterioles, tubular atrophy, interstitial fibrosis, and glomerular sclerosis, with the hallmark feature being the arteriolar lesion.[5] It has been speculated that the non-vascular renal changes, which are said to have a "stripe-like" distribution, are due, at least in part, to ischemia resulting from the renal arteriolar changes.[4,5] Similarly, the arteriolar changes are also thought to be responsible for the associated hemolytic anemia because they result



1-4. Kidney, cynomolgus macaque: Glomeruli are enlarged and contain increased amounts of eosinophilic protein surrounding glomerular capillaries (membranous glomerulonephritis). (HE 320X)

in mechanical damage and eventual intravascular hemolysis of red blood cells.[2]

This case was complicated by the fact that this animal also had a mononuclear meningitis, suggestive of a viral etiology (although no viral inclusion bodies were noted microscopically) secondary to cyclosporine-induced immunosuppression. Primary differentials for meningoencephalitis include simian virus 40 (SV-40), which can also cause interstitial nephritis,[6] and herpesviral infection, specifically cytomegalovirus (CMV).[1]

Immunohistochemistry on affected areas of brain was attempted and was negative for SV-40, but CMV antibodies were not reactive against macaque CMV.

JPC Diagnosis: 1. Kidney, arterioles: Arteritis, proliferative, diffuse, moderate.
2. Kidney, tubules: Degeneration and necrosis, multifocal, mild with tubular ectasia and protein casts.
3. Kidney: Glomerulonephritis, membranous, diffuse, moderate.

Conference Comment: Cyclosporine and tacrolimus are often used in conjunction with human kidney transplants in an attempt to prevent chronic allograft rejection. The immunosuppressive properties associated with these drugs result from inhibition of calcineurin (a calcium- and calmodulin-dependent phosphatase protein), with subsequent reduction in the transcription of T-cell-dependent lymphokines, such as interleukin-2 (IL-2), interferon-γ (IFN-γ, tumor necrosis factor-α (TNF-α), and granulocyte macrophage colony-stimulating factor (GM-CSF); these are regulated through calcineurin-dependent dephosphorylation of NFAT. The selective immunosuppressive properties of calcineurin inhibitors (CNIs) are attributed to the localization of NFAT predominantly within T-lymphocytes; however, long-term administration may result in toxic changes, specifically nephrotoxicity.[8-10]

Acute CNI nephrotoxicity results from vasoconstriction of the afferent arteriole due to increased levels of vasoconstrictors (such as endothelin, thromboxane and activation of the renin-angiotensin system) and decreased quantities of vasodilators (such as prostaglandin-E2, prostacyclin, and nitric oxide). This dose-dependent and reversible arteriolar vasoconstriction results in reduced renal blood flow, reduced glomerular filtration, increased renal vascular resistance and tubular dysfunction.[8] Chronic CNI nephrotoxicity is also associated with impairment of renal function and is likely mediated by several different growth factors and cytokines, including transforming growth factor beta (TGFβ), platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and TNF-α. CNIs have been shown to produce various side effects in addition to nephrotoxicity, including diabetes, neurological dysfunction, hemolytic anemia, hemolytic uremic syndrome, hypertension and hypercholesterolemia.[2,9,10]

Although there is significant tubular degeneration/regeneration as well as mild glomerulonephritis and glomerulosclerosis, conference participants agree that the most striking lesions in this interesting case are the occlusive arteriolar changes, consisting of disruption of the internal elastic membrane, proliferation, hypertrophy, hyalinosis and fibrosis within the tunica intima/media and significant narrowing of affected lumina. Similar changes are reported in association with chronic allograft nephropathy (characterized by progressive and irreversible deterioration of renal function with interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, and glomerulosclerosis) and systemic hypertension.[8] In addition to the microscopic lesions described above, which are consistent with cyclosporine nephrotoxicity, there is evidence of

microangiopathic hemolytic anemia, supported by the finding of regenerative anemia on the CBC and the detection of schistocytes, which are fragmented erythrocytes suggestive of shearing of erythrocyte membranes, on the peripheral blood smear. Microangiopathic hemolytic anemia is another well-described sequela to administration of CNIs,[2,9] presumably secondary to erythrocyte damage from the turbulence that results from vasoconstriction and occlusion of renal arterioles. Although further clinical-pathologic data is not available, hemoglobinemia and hemoglobinuria, as well as hyperbilirubinemia, are also expected findings in cases of microangiopathic anemia.[11]

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CASE II: 13-226/227 (JPC 4033976).

Signalment: Two age-unknown (post-weaning) female and castrated male Norwegian Landrace pigs, (*Sus scrofa domesticus*).

History: Two pigs were received for necropsy from a farm with diarrhea among weaned pigs.

Gross Pathology: The nutritional status of both pigs was slightly below normal. Feces in the large bowel of both pigs had looser consistency than normal, but the cecum and colon were otherwise normal macroscopically. One of the pigs had slightly enlarged mesenteric lymph nodes.

Laboratory Results: From colons of both pigs a mixed bacterial growth with *Brachyspira sp.* was cultured and from both pigs the *Brachyspira sp.* were identified as *Brachyspira pilosicoli.*

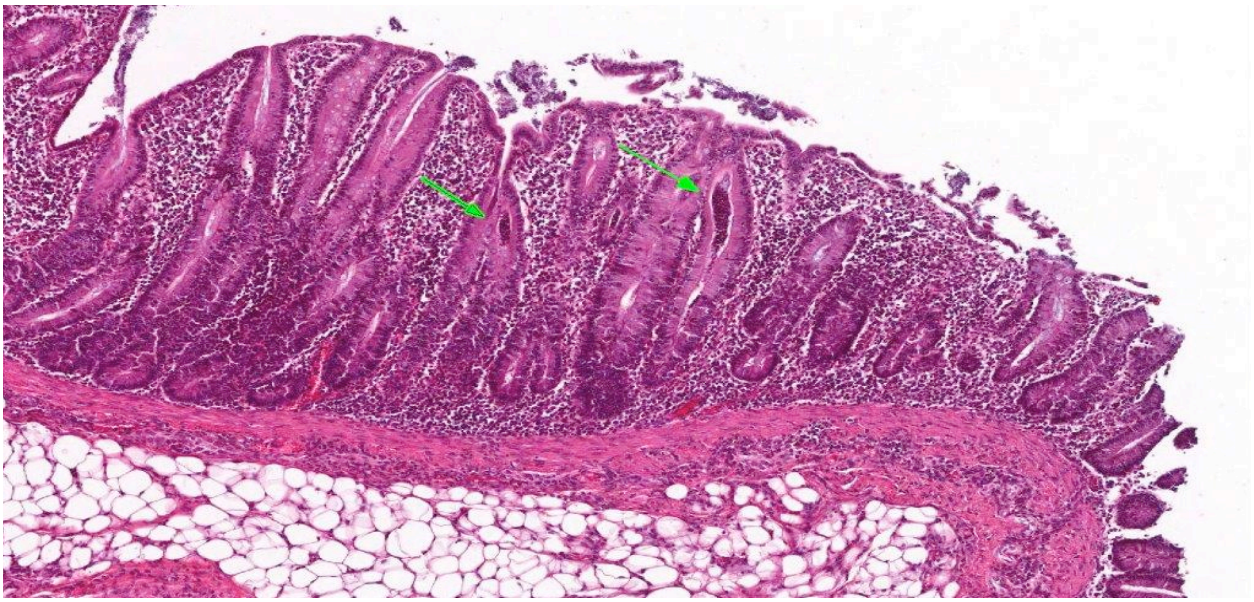
Histopathologic Description: Histologic findings were similar in colon and cecum from one pig (13/226) and colon from the other pig (13/227). Surface epithelium was multifocally attenuated, and variably covered by numerous bacteria. Numerous crypts contained abundant bacteria with a morphology consistent with spirochetes. Number of goblet cells in crypt epithelium varied from few to moderate, but was generally decreased. Some crypts contained abundant neutrophils (crypt abscesses), and some crypts were lined by flattened attenuated epithelial cells. The lamina propria contained a moderate increase in lymphocytes and macrophages and few neutrophils.

In the deeper parts of some crypts (moderate numbers in the colon of pig 13/226, and few in the cecum from pig 13/226 and colon from pig 13/227) there were luminal pyriform to crescent-shaped organisms, approximately 5 x 7 µm in size, with a faint nucleus and eosinophilic cytoplasm (consistent with trichomonads).

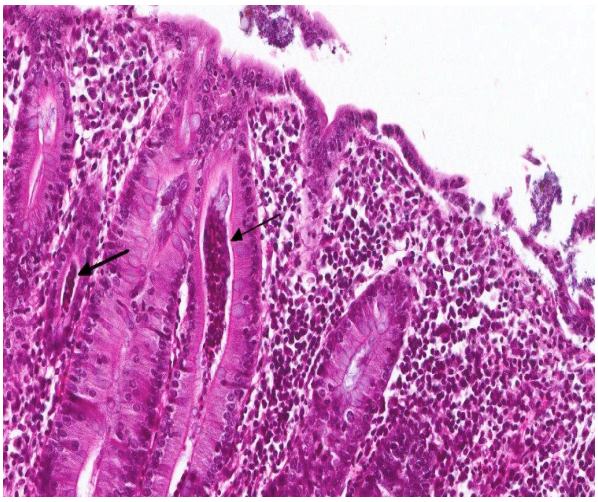
In a few sections there were also a few ciliated large protozoa (*Balantidium coli*) on the surface of the mucosa (incidental finding).

Contributor's Morphologic Diagnosis: Cecum and colon: Typhlocolitis, catarrhal, diffuse, mild with moderate numbers of crypt abscesses, high numbers of intracryptal spirochetes and moderate numbers of intracryptal trichomonads and few surface ciliated large protozoa.

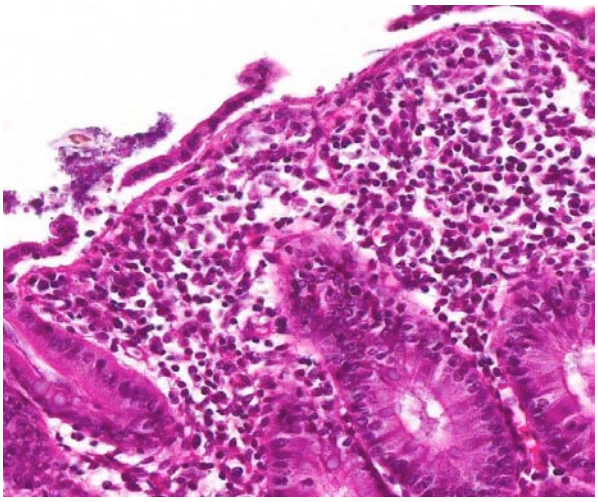
Contributor's Comment: Two members of the genus *Brachyspira* may cause colitis in swine: the strongly hemolytic *Brachyspira hyodysenteriae* which is the cause of swine dysentery, and the weakly hemolytic *B. pilosicoli* which is the cause of porcine intestinal spirochetosis.[1,3] *B. pilosicoli* has a wide host range, is capable of infecting a



2-1. Colon, pig: The lamina propria is markedly expanded by numerous inflammatory cells including neutrophils and lymphocytes, and there are occasional crypt abscesses. (HE 160X)



2-2. Colon, pig: Higher magnification of Fig 2-1 with dilated glands (arrows) filled with neutrophils, degenerate epithelial cells, and occasional trichomonads. (HE 240X)



2-3. Colon, pig: The overlying epithelium is multifocally infiltrated by inflammatory cells, often detached, and multifocally covered by mats of robust bacilli. (HE 400X)

number of animal species, both mammals and birds, and may also be zoonotic.[1] Other weakly hemolytic members of the *Brachyspira* genus that may be isolated from swine, *B. innocens, B. intermedia* and *B. murdochii*, have not been shown to cause disease in experimentally infected conventional pigs and are considered to be nonpathogenic commensals in pigs.[3] While swine dysentery is a highly infectious disease of mainly weaned pigs characterized by a large bowel diarrhea with mucus, blood, or fibrin in the feces, porcine intestinal spirochetosis is typically a milder disease characterized by transient watery to mucoid diarrhea without blood,[1] as was seen in these two pigs. The histologic findings are focal erosions, slight edema, crypt abscesses, mild infiltrate of mononuclear cells in lamina propria and the spirochetes may be found attached to surface epithelium, inside dilated crypts, invading through tight junctions between colonic enterocytes, within goblet cells, and within the lamina propria.[3,4]

The protozoal organisms located in colonic crypts were interpreted as trichomonads. *Tritrichomonas suis* is considered to be an apathogenic commensal in pigs that may colonize nasal cavity and intestines of pigs. This species has been found to be identical in sequence to *T. foetus*,[2,7] a venereally transmitted pathogen that affects cattle; however, other trichomonads may also be identified in fecal samples from pigs, such as *Tetratrichomonas buttreyi,*[6] *Trichomitus rotunda* and others unrelated to previously described trichomonads.[5]

JPC Diagnosis: Colon: Colitis, necrotizing, subacute, diffuse, moderate, with marked crypt hyperplasia.

Conference Comment: Conference participants conducted a brief discussion of the differential diagnosis for the clinical, gross and histological findings in this case, including *Brachyspira hyodysenteriae, Lawsonia intracellularis, Salmonella* spp., *Clostridium* spp., *Escherichia coli*, *Trichuris suis*, coronavirus and rotavirus. As noted by the contributor, *B. hyodystenteriae* is strongly beta-hemolytic and highly infectious, causing severe hemorrhagic diarrhea and fibrinonecrotic pseudomembranous colitis in affected swine. Although virulence factors are poorly defined, both *B. dysenteriae* and *B. pilosicoli* are thought to be chemotactically attracted to intestinal mucin and tend to be intimately associated with the intestinal mucus layer. *Lawsonia intracellularis*, on the other hand, is an obligate intracellular gram-negative bacterium that colonizes enterocytes in the ileum and colon, resulting in proliferative to necrotizing enteritis/colitis. *Salmonella* spp. are important enteric pathogens of swine. *S. typhimurium* causes acute/chronic enterocolitis in feeder pigs; it has also been associated with necrotizing proctitis and subsequent rectal stricture. The major clinical manifestation of *S. cholerasuis* is septicemia with secondary endothelial damage. Common microscopic findings include interstitial pneumonia, multiple foci of hepatic necrosis ("paratyphoid nodules"), renal cortical microhemorrhages ("turkey egg" kidney),

polyarthritis or polysynovitis, meningoencephalomyelitis and enterocolitis, often with colonic "button ulcers."[1]

C. perfringens type C is associated with outbreaks of necrohemorrhagic enteritis in suckling piglets, while *C. difficile* is recognized as a cause of fibrinous typhlocolitis and mesocolonic edema in neonatal piglets (see WSC 2013-2014, conference 17, case 1 for a more detailed discussion of *Clostridium* spp.). Enterotoxigenic *E. coli* (ETEC) causes profuse watery diarrhea in neonatal piglets via heat-labile (LTI and LTII) and heat-stable (STa and STb) enterotoxins (see WSC 2013-2014, conference 12, case 2 for a more detailed discussion of *E. coli*); however, histological changes are typically minimal. Enteropathogenic (attaching and effacing) *E. coli* is a less frequently reported cause of diarrhea in swine, and, although an uncommon manifestation, enteritis has also been associated with some strains of enterohemorrhagic, Shiga-like toxin producing *E. coli*, the cause of porcine edema disease. The whipworm *Trichuris suis* inhabits the cecum and colon of swine, where it embeds in the surface epithelium; severe infections may lead to mucohemorrhagic typhlocolitis.[1]

There are two coronaviruses associated with porcine diarrhea. Transmissible gastroenteritis (TGE) is a highly contagious disease that is most severe in piglets younger than 10 days of age, characterized by high morbidity with vomiting and profuse diarrhea. The causative agent, a group 1 porcine coronavirus, destroys intestinal villar epithelial cells, resulting in marked villar atrophy and diarrhea secondary to malabsorption. Porcine epidemic diarrhea (PED) virus is another group 1 coronavirus that causes similar, though less severe signs in older pigs. The PED virus is endemic in many Asian countries, and has been present in Europe since the early 1970s; however, until recently (i.e., May 2013), it was not present in the United States, and its emergence has caused epidemic disease with important economic implications for the American pork industry. Rotavirus is enzootic in many swine herds and causes villar atrophy with ensuing diarrhea in both suckling and weaned pigs, although it is generally less severe than TGE. Finally, porcine adenoviral inclusions have been identified within small intestinal enterocytes in both asymptomatic pigs and those presenting with watery diarrhea; however, the significance of this finding remains controversial, and adenoviral infection is not yet recognized as a significant cause of enteric disease in swine.[1]

In this case, histochemical staining with Warthin-Starry highlights numerous argyrophilic spirochetes adhered to the enteric mucosa, supporting a diagnosis of colonic spirochetosis, while the culture results as reported by the contributor definitively identify the etiologic agent as *Brachyspira pilosicoli*.

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References:

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CASE III: AFIP Case 2 (JPC 3165069).

Signalment: 3-year old male Boer goat, (*Capra hircus*).

History: Per referring veterinarian: This goat presented with a severe anemia due to parasitism (9% hematocrit), and a fresh, whole blood transfusion was administered. Four hours after transfusion, the goat developed an increased respiratory rate and open-mouthed breathing. Treatments with anti-inflammatory drugs, anti-bacterial drugs, and thoracocentesis for hydrothorax did not ameliorate clinical signs, and the goat died three hours later.

Gross Pathology: Within the thoracic cavity are approximately 700 ml of clear, watery, yellow fluid. Lungs are diffusely rubbery and mottled red and tan with few foci of atelectasis. The pericardial sac contains approximately 70 ml of fibrinous exudate.

The mediastinal lymph nodes are expanded by abundant amounts of caseous material. Scattered throughout the liver and spleen are dozens of variably-sized abscesses. The kidneys are slightly tan to grey, and the subcapsular surface is slightly granular and mottled with pinpoint, tan foci.

Laboratory Results: *Corynebacterium pseudotuberculosis* was cultured from the hepatic and splenic abscesses. Fecal floatation revealed a trichostongyle egg count of 3,400.

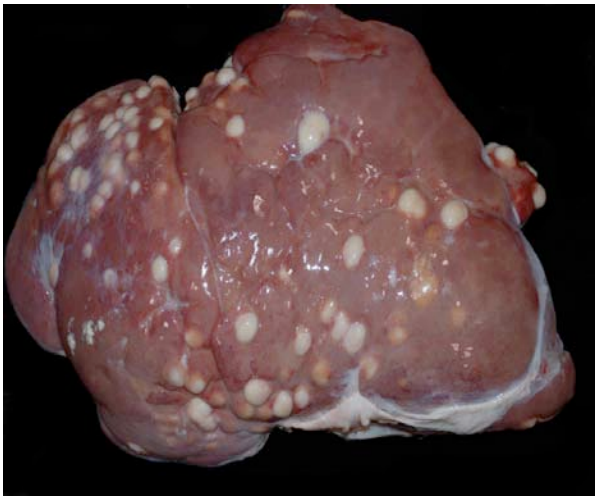
Histopathologic Description: On histology, the majority of hepatic tissue is replaced by multifocal, large abscesses that compress hepatic parenchyma and distort hepatic lobules. Abscesses are composed of large central aggregates of necrotic cellular debris infiltrated by many neutrophils and surrounded by a thick fibrous capsule consisting of well-organized fibroblasts admixed with lymphocytes and occasional bile ducts. In the surrounding hepatic tissue, portal triads are markedly expanded by proliferative bile ducts, admixed with large aggregates of amyloid and many lymphocytes, plasma cells, and macrophages.

Within the spleen are numerous large abscesses, and surrounding periarteriolar lymphoid aggregates are large aggregates of amyloid. Amyloid distends glomerular tufts. Histochemical staining with Congo red confirms the presence of amyloid as congophilic material that is birefringent and apple green on polarization.

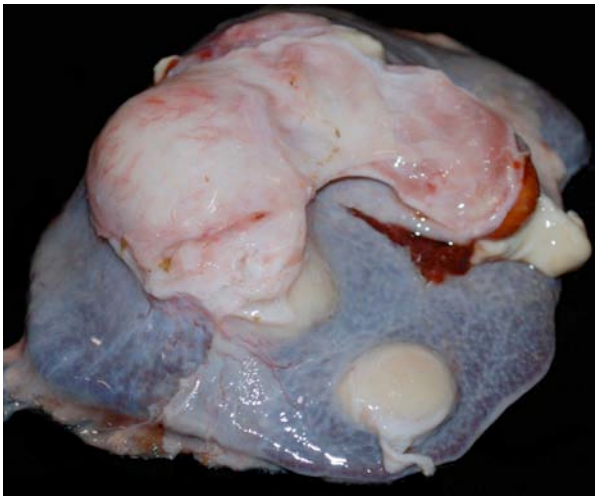
Histologic examination of the lungs revealed an acute, neutrophilic interstitial pneumonia.

Contributor's Morphologic Diagnosis: Liver: Severe, multifocal, chronic hepatic abscesses with marked portal fibrosis, biliary hyperplasia, and portal amyloidosis.

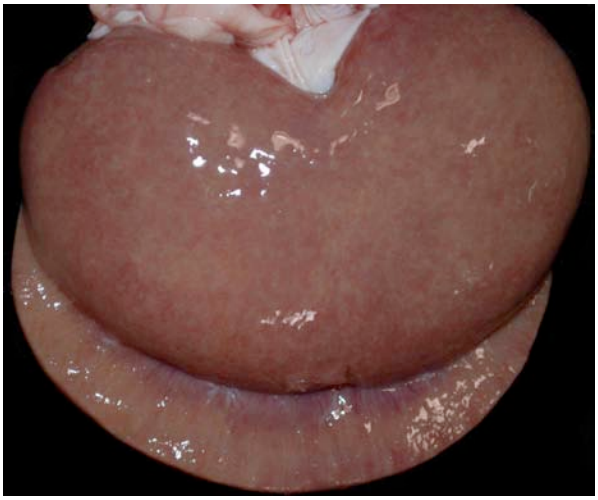
Contributor's Comment: Multiple abscesses in the liver, spleen, and lymph nodes were due to



3-1. Liver, Boer goat: Variably sized abscesses are distributed randomly throughout all lobes of the liver. (Photo courtesy of: Oklahoma State University Department of Veterinary Pathobiology, Room 250 McElroy Hall, Stillwater, OK 74078 www.cvm.okstate.edu)



3-2. Spleen, Boer goat: Variably sized abscesses are distributed randomly throughout the spleen. (Photo courtesy of: Oklahoma State University Department of Veterinary Pathobiology, Room 250 McElroy Hall, Stillwater, OK 74078 www.cvm.okstate.edu)

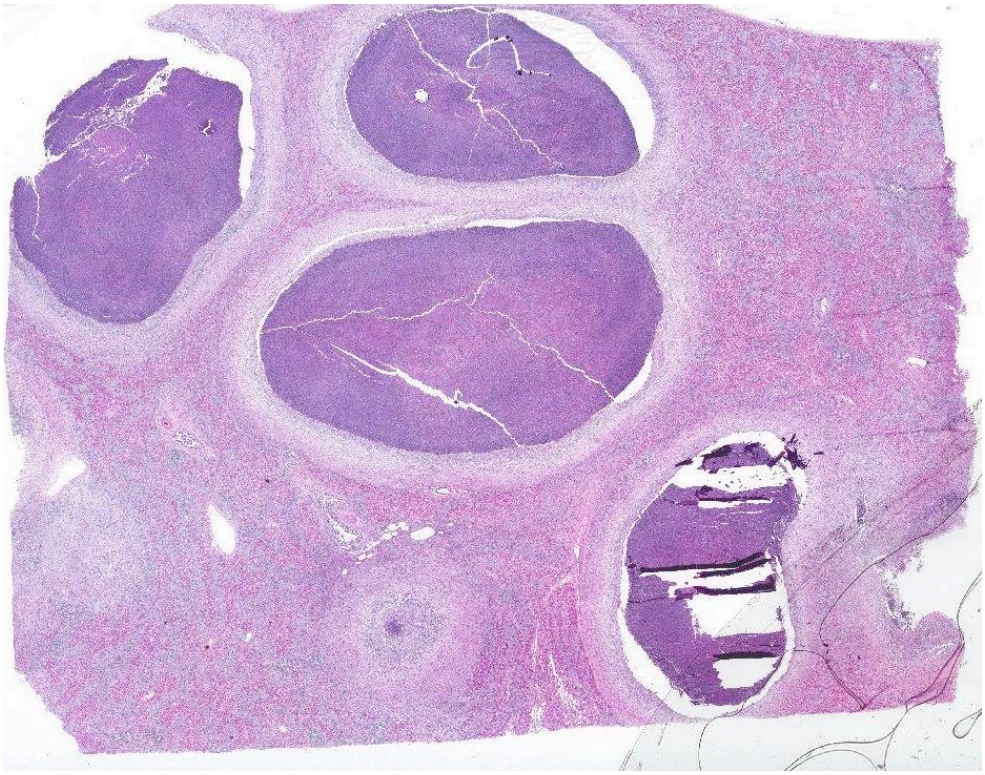


3-3. Kidney, Boer goat: The kidneys are slightly enlarged and a mottle tan-grey color with numerous pinpoint tan foci. (Photo courtesy of: Oklahoma State University Department of Veterinary Pathobiology, Room 250 McElroy Hall, Stillwater, OK 74078 www.cvm.okstate.edu)

infection with *Corynebacterium pseudotuberculosis*. As a result of chronic inflammatory disease, the goat developed secondary systemic amyloidosis. Death was attributed to the acute pneumonia, possibly due to a transfusion reaction.

Corynebacterium pseudotuberculosis is a gram-positive, pleomorphic, facultative, anaerobic bacillus that commonly causes disease in several domestic species, including goats, sheep, cattle, and horses. In small ruminants, *C. pesudotuberculosis* is a common cause of lymphadenitis (caseous lymphadenitis), and it may cause pectoral muscle abscesses in horses (pigeon fever) and ulcerative lymphangitis in cattle. In addition, *C. pseudotuberculosis* may cause subcutaneous abscesses, splenic abscesses, embolic nephritis, and orchitis.[6] As in this case, C. *pseudotuberculosis* infection is occasionally associated with systemic amyloidosis in small ruminants.[4,7]

Systemic amyloidosis refers to the deposition of amyloid in multiple organs, as opposed to localized amyloidosis, where amyloid is deposited in a single organ. Systemic amyloidosis may result from an immunocyte dyscrasia (primary systemic amyloidosis) or from chronic inflammation (secondary systemic amyloidosis). Amyloid deposited in secondary systemic amyloidosis is composed of AA (amyloid-associated) protein that forms β-pleated sheets. AA protein is derived from serum amyloid-associated (SAA) protein which is produced by the liver as an acute phase reaction to inflammation.[6]



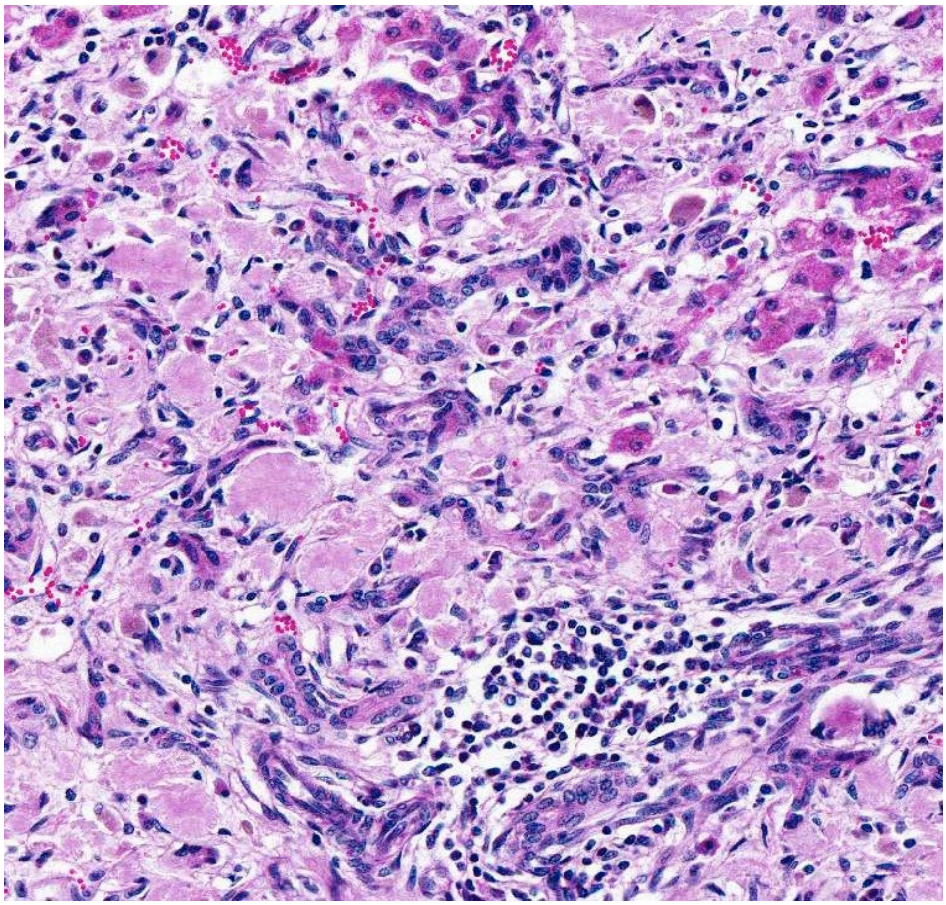
3-4. Liver, Boer goat: The section contains multiple discrete abscesses measuring up to a centimeter in diameter. (HE 14X)

In small ruminants, secondary systemic amyloidosis has been reported to most commonly result from pneumonia,[5,7] though it may also occur in association with nephritis,[5] polyarthritis, urolithaisis, and mastitis.[7] Typically, small ruminants with secondary systemic amyloidosis may have deposits of amyloid in the kidneys, spleen,

liver, lymph nodes, gastrointestinal tract, adrenal gland, and vascular tunica media.[2,7]

As in this case, renal glomeruli may be more effected than the renal medulla, and amyloid may form aggregates surrounding splenic periarteriolar lymphoid aggregates.[7] In contrast to this case, hepatic amyloidosis in small ruminants, is more commonly associated with expansion of the sinusoids rather than the portal triads.[4,7]

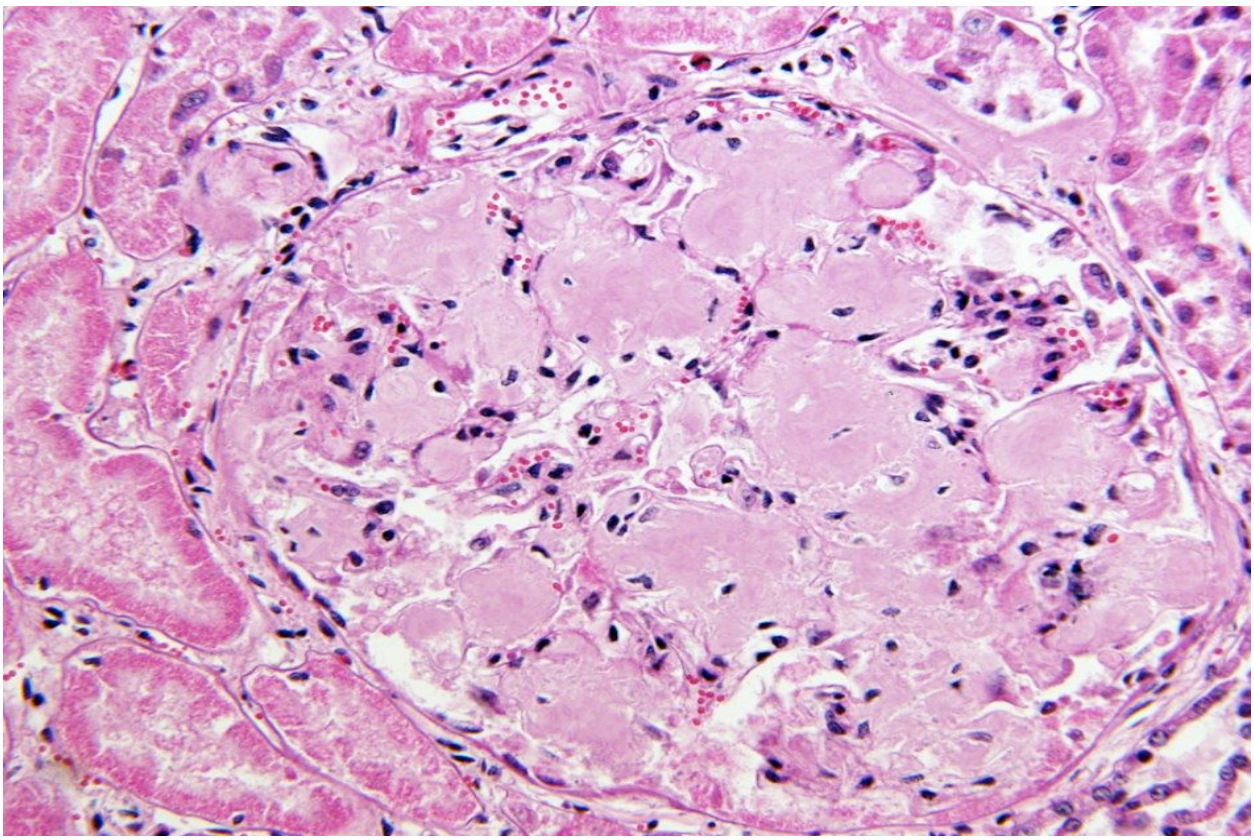
JPC Diagnosis: Liver: Abscesses, multiple, with marked hepatocellular fibrosis, hepatocellular atrophy and loss, biliary hyperplasia, and amyloid formation.



3-5. Liver, Boer goat: Throughout the remaining liver, hepatic sinusoids and portal triads are expanded by abundant amyloid which compresses adjacent hepatocytes. There is marked ductular reaction. (HE 220X)

Conference Comment: There is some slide variation in this case, and several conference participants described multiple hepatic abscesses while others identified the lesions as pyogranulomas. Participants further noted numerous small, duct-like structures composed of cuboidal cells within the hepatic parenchyma, which prompted a focused exploration of the distinction between bile duct hyperplasia and ductular reaction. Although the liver maintains the ability to "regenerate" via a tightly controlled process of compensatory hyperplasia, some types of damage, such as toxic injury and massive hepatic necrosis, may render hepatocytes unable to multiply. Instead, there is proliferation of progenitor cells (oval cells), which often form vague, poorly-differentiated ductular structures that are connected to individual canals of Hering and eventually differentiate into hepatocytes or cholangiocytes. This phenomenon, known as ductular reaction, occurs within the hepatic parenchyma.[3] Conversely, bile ductular hyperplasia and proliferation (as seen in cases of bile duct obstruction) are characterized by piling up of epithelium, micropapillary projections, and/or luminal enlargement or distortion, while tortuous bile ducts manifest histopathologically as increased ductular profiles; these changes are generally confined to portal tracts.[8]

Based on the microscopic findings in this case, *Fusobacterium necrophorum* was suggested as a possible etiologic agent. Hepatic necrobacillosis in ruminants typically occurs when this opportunistic, gram-negative bacterium enters portal circulation as a sequela to toxic rumenitis.[6] Gram-negative septicemia, due to agents such as *E. coli* or *Salmonella* spp., could also result in hepatic abscessation. In this case, a Masson's trichrome and a Gram stain reveal thick connective tissue capsules surrounding multiple abscesses containing numerous gram-positive bacilli. This, in combination with the culture results as reported by the contributor, identifies *C. pseudotuberculosis* as the underlying cause.



3-6. Kidney, Boer goat: Glomerular tufts are markedly expanded by amyloid. (HE 400X) (Photo courtesy of: Oklahoma State University Department of Veterinary Pathobiology, Room 250 McElroy Hall, Stillwater, OK 74078 www.cvm.okstate.edu)

The contributor provides an excellent summary of both *Corynebacterium pseudotuberculosis* (which is a potential zoonosis) and systemic amyloidosis in ruminants. Although *C. pseudotuberculosis* is relatively poorly characterized, some virulence determinants include the following: the leukotoxic phospholipase D exoprotein (PLD) contributes to the destruction of caprine macrophages during infection; the fagABC operon and the fagD gene play a role in virulence and are involved in iron acquisition; the high cell wall concentration of lipids aids in resistance to enzymatic digestion, allowing the bacterium to persist as a facultative intracellular parasite; and CP40, an immunogenic protein that exhibits proteolytic activity as a serine protease.[9] Additionally, recent studies have demonstrated that serum concentrations of haptoglobin (Hp), serum amyloid A and α1 acid glycoprotein are increased in experimental models of ovine caseous lymphadenitis due to *C. pseudotuberculosis*, which may predispose affected animals to systemic amyloidosis.[1] Readers are urged to review WSC 2013-2014, conference 6, case 4 for a more detailed discussion of amyloidosis.

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CASE IV: NEPRC Case 2 (JPC 3163069).

Signalment: 5-year-old male intact rhesus macaque, (*Macaca mulatta*).

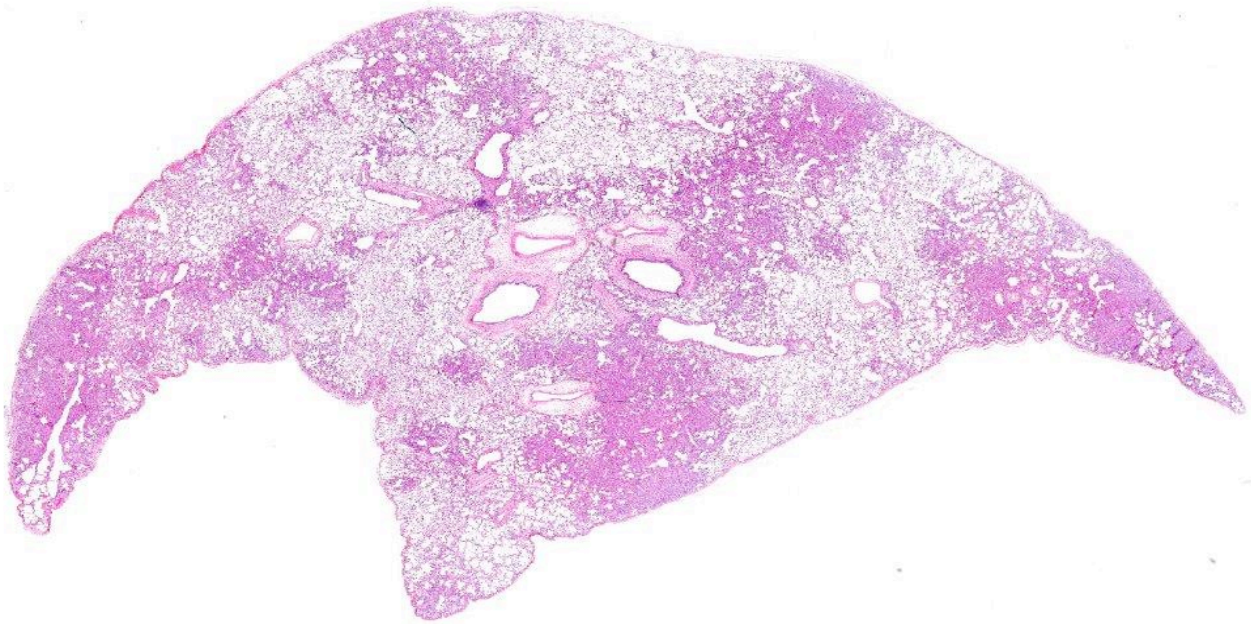
History: This monkey was inoculated with SIVmac251 and was started on combined anti retroviral treatment (CART) 2 months following inoculation. CD 8 depletion was performed on days 6, 8, and 12 post infection.

Gross Pathology: The animal was in thin body condition and alopecia was present over the neck and back. The lungs were mottled, firm and partially collapsed with the left more affected than the right. The small intestine was multifocally thickened.

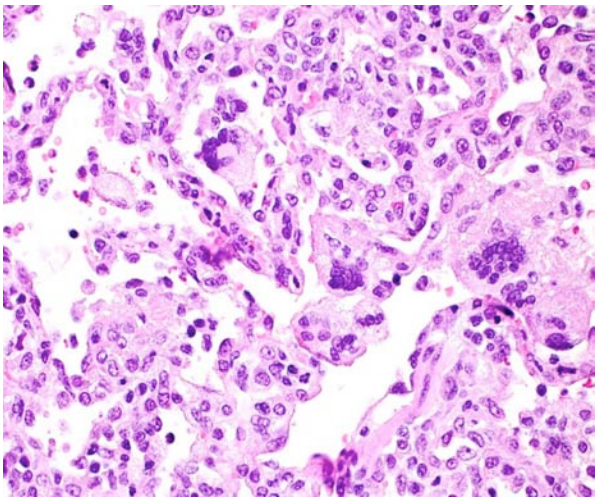
Histopathologic Description: Lung: Within the lung parenchyma, there are multifocal to coalescing areas (50 % of total area) of inflammation composed of a mixed population of foamy macrophages, histiocytes, multi-nucleated giant cells, and few numbers of neutrophils, lymphocytes, and plasma cells. Interspersed with these areas of inflammation is marked type II pneumocyte hyperplasia that often protrudes into and obscures affected alveolar lumina. Additionally, alveoli frequently contain foamy macrophages that are admixed with proteinaceous fluid (edema). The multinucleate cells are abundant in many areas and often contain up to 25 irregularly spaced nuclei. Often interspersed with the areas of inflammation are aggregates of non-degenerate neutrophils and macrophages in which the macrophages often are cytomegalic with marked karyomegaly and intranuclear eosinophilic inclusion bodies that measure 15-25 µm in diameter and are surrounded by a clear halo. Within large airways are occasional plugs of mucus, degenerate cellular debris, and scattered degenerate neutrophils. The pleura is multifocally thickened by fibrosis in several areas.

Contributor's Morphologic Diagnosis: Lung: Multifocal to coalescing severe chronic neutrophilic and histiocytic interstitial pneumonia with giant cells and intranuclear cytomegalovirus inclusions.

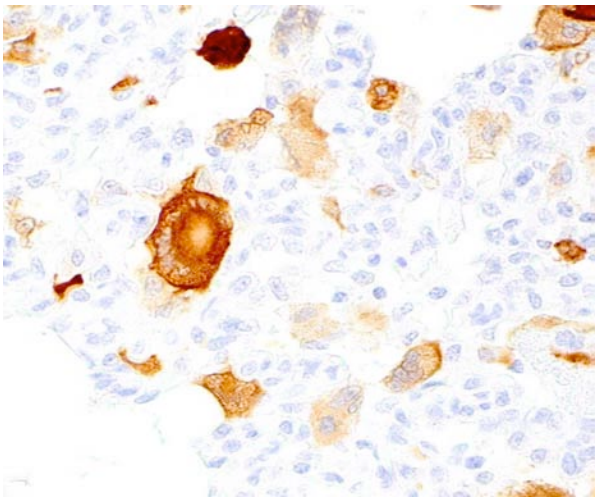
Contributor's Comment: Simian acquired immunodeficiency syndrome (SAIDS) is characterized by chronic persistent infection with simian immunodeficiency virus (SIV) along with high viremia, low CD4 titer as well as low anti-SIV antibodies that results in opportunistic infections.[14] These opportunistic infections include cytomegalovirus (CMV), *Pneumocystis*, *Mycobacterium* and adenoviral infections.[1]



4-1. Lung, rhesus macaque: Approximately 50% of the lung exhibits patchy consolidation. (HE 0.63X)



4-2. Lung, rhesus macaque: Within consolidated areas, alveolar septa are expanded by hyperplastic type II pneumocytes, macrophages, edema, and alveoli contain numerous foamy macrophages, multinucleated viral syncytia, and fewer neutrophils. (HE 400X) (Photo courtesy of: New England Primate Research Center, Harvard Medical School, One Pine Hill Dr., Southborough, MA 01772 http://www.hms.harvard.edu/nerprc)



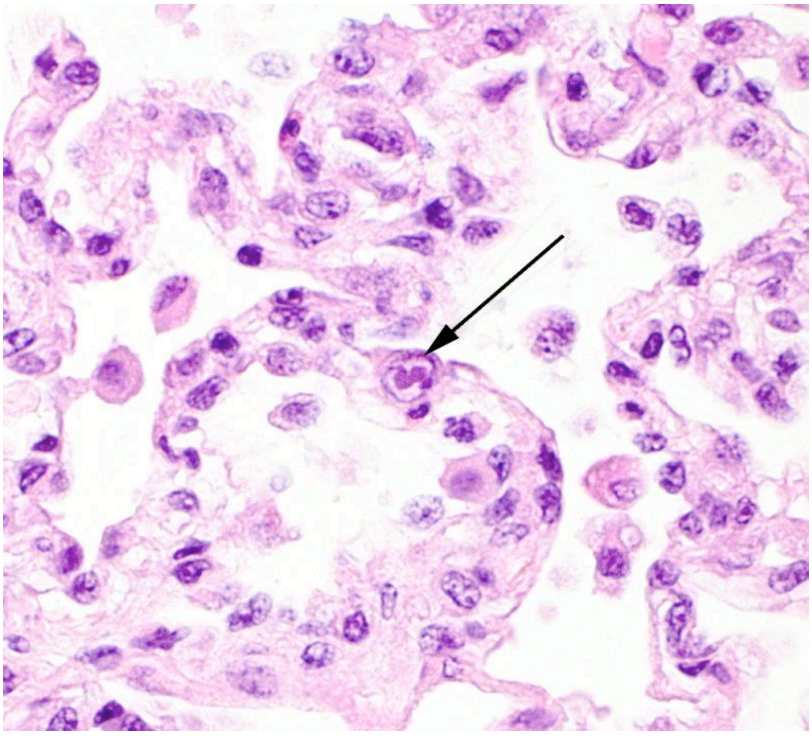
4-3. Lung, rhesus macaque: Giant cell macrophages are immunopositive for simian immunodeficiency viral antigen. (400X) (Photo courtesy of: New England Primate Research Center, Harvard Medical School, One Pine Hill Dr., Southborough, MA 01772 http://www.hms.harvard.edu/nerprc)

SIV belongs to genus lentivirus under the family *Retroviridae*. Retrovirus virions are enveloped, 80-100 nm in diameter, and have a unique three-layered structure. Innermost is the genome nucleoprotein complex, which includes about 30 molecules of reverse transcriptase, and has helical symmetry. This structure is enclosed within an icosahedral capsid, about 60 nm in diameter, which in turn is surrounded by a host cell membrane-derived envelope from which glycoprotein peplomers project. The genome is diploid, consisting of an inverted dimer of two molecules of linear positive-sense, single stranded RNA; each monomer is 7-11 kb in size and has a 3'-polyadenylated tail and a 5'-cap; the retrovirus genome is organized into 9 ORFs producing 15 proteins. SIV infection causes a spectrum of virally-induced lesions that include enteropathy, lymphocytic interstitial pneumonitis, giant cell pneumonia/lymphadenitis, and SIV encephalitis.

Giant cell pneumonia is characterized by multinucleated giant cells that are frequently found in terminal cases of SAIDS.[3] Formation of multinucleated giant cells is not completely defined but requires a myriad of factors including 1) infection with SIV, 2) cytokine elaboration from the multinucleated giant cells, and 3) macrophage infiltration.[2]

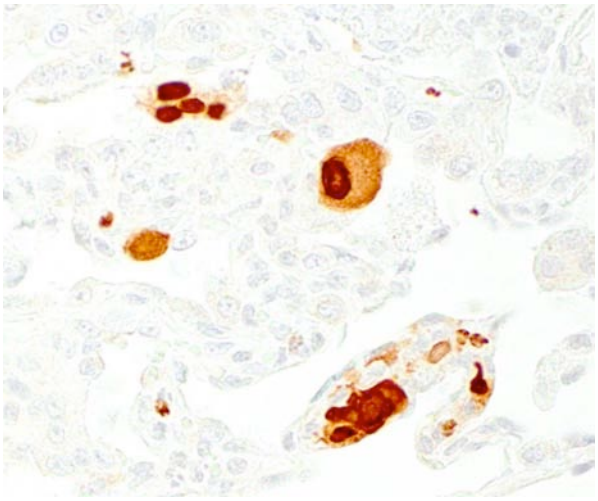
Cytomegalovirus (CMV) virions are enveloped, about 150 nm in diameter, and contain an icosahedral nucleocapsid about 100 nm in diameter. The genome consists of a single linear molecule of double-stranded DNA, 125-235 kbp in size. CMV belongs to the *Betaherpesvirinae* subfamily of *Herpesviridae,* under the order *Herpesvirales*. There are number of cytomegalovirus isolated from non-primates that include Cercopithecine herpesvirus 5 (CeHV-5) in African green monkeys, Cercopithecine herpesvirus 8 (CeHV-8) in rhesus monkeys, Human herpesvirus 5 (HHV-5) in humans, as well as Pongine herpesvirus in chimpanzees, Aotine herpesvirus 1 & Aotine herpesvirus 3 in owl monkeys. Betaherpesviruses replicate more slowly than alphaherpesviruses and often produce greatly enlarged cells, hence the designation cytomegalovirus.[16] Cytomegaloviruses infect the salivary glands, liver, spleen, lungs, eyes, and other organs, in which they produce characteristically enlarged cells with intranuclear inclusions and are typically accompanied by neutrophilic infiltrates.

Another common opportunistic lung infection in immunocompromised rhesus macaques is *Pneumocystis*. *Pneumocystis* pneumonia (PCP) is one of the most common opportunistic diseases in SAIDS.[1,2] Characteristic pathologic features of PCP include infiltration of inflammatory cells in the lung interstitium, thickened alveolar septa by hyperplastic type II pneumocytes, and foamy exudates in the alveoli. Some of these features



4-4. Lung, rhesus macaque: Within consolidated areas, occasional pneumocytes are karyomegalic and the nucleus contains a single enlarged eosinophilic cytomegaloviral inclusion. (400X) (Photo courtesy of: New England Primate Research Center, Harvard Medical School, One Pine Hill Dr., Southborough, MA 01772 http://www.hms.harvard.edu/nerprc)

were present in the submitted case; however, immunohistochemistry for *Pneumocystis* was negative in this case. Since *Pneumocystis* has a morphology similar to protozoa, it was initially considered as such; however, it is now classified as a fungus because the composition and structure of its cell wall [12,15] and nucleotide sequences are more similar to those of fungi than to those of protozoa.[6,13] Although *Pneumocystis* organisms are found in many different species of mammals, they are strictly species specific.[7] Some of the more common organisms include: *Pneumocystis jirovecii* (human), *P. wakefieldii* (rat), *P. murina* (mouse), and *P. carinii* (rhesus macaque).[5] In immunocompetent humans and animals, alveolar macrophages (AMs) protect the hosts against *Pneumocystis* infection by actively removing this extracellular organism from the alveoli. However, AMs from Pneumocystis infected animals are defective in phagocytosis,[4,9] and the number of AMs in humans and animals with PCP is reduced. These two defects impair innate immunity against *Pneumocystis* infection. The defect in phagocytosis is correlated to down regulation of mannose receptor on the macrophages.[8] The reduction in alveolar macrophage (AM) number is mainly due to increased rate of apoptosis[10] that is triggered by increased levels of intracellular polyamines[11] which could be due to either increased de novo synthesis and uptake of exogenous polyamines. Very little is known about the defect in phagocytosis during PCP.



4-5. Lung, rhesus macaque: Pneumocytes are rarely immunopositive for cytomegalovirus antigen. (400X) (Photo courtesy of: New England Primate Research Center, Harvard Medical School, One Pine Hill Dr., Southborough, MA 01772 http://www.hms.harvard.edu/nerprc)

JPC Diagnosis: 1. Lung: Pneumonia, interstitial, histiocytic, with numerous viral syncytial giant cells.
2. Lung, alveolar macrophages: Intranuclear viral inclusions, rare.

Conference Comment: We thank the contributor for providing this excellent example and thorough analysis of two important entities in non-human primates. As noted by the contributor and demonstrated in this case, giant-cell pneumonia is a pathognomonic condition associated with SIV infection, while secondary infection with CMV produces significantly enlarged cells with characteristic intranuclear inclusion bodies. Although neutrophilic infiltrates typically accompany CMV infection, they are not a prominent feature in this case. The moderator also pointed out several opportunistic infections

(in addition to cytomegalovirus, *Pneumocystis* spp. mycobacteriosis, and adenovirus) associated with SIV infection in non-human primates, including *Cryptosporidium* spp., *Shigella* spp., *Campylobacter* spp. and Epstein-Barr virus.

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