Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2014-2015

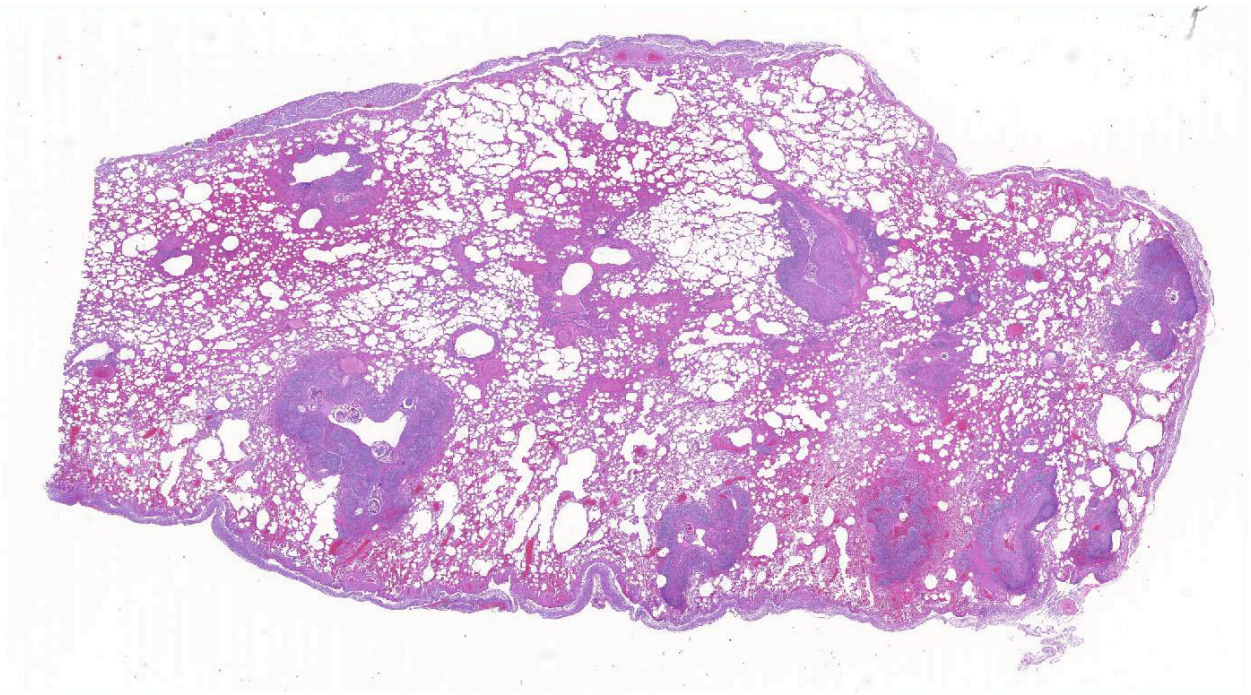
Conference 6

8 October 2014

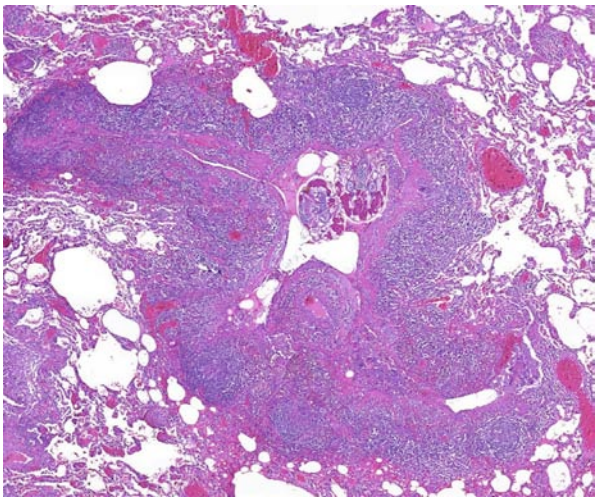
CASE I: NHP 14-22 (JPC 4048655).

Signalment: 10-year-old intact male rhesus macaque, *(Macaca mulatta)*.

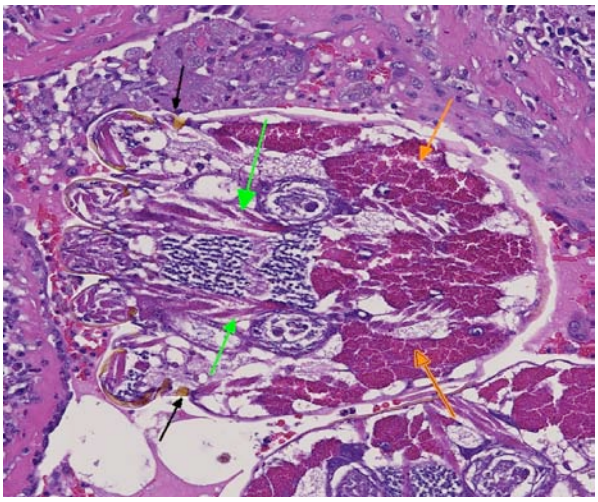
History: This animal was part of a study at an outside research institution that was looking at neural recordings. The day after the first recording was made from the deep brainstem, vestibular signs were noted. The animal was treated and



1-1. Lung, rhesus macaque: Airways are prominent as a result of ectasia and a prominent mural cellular infiltrate. There is a cellular exudate overlying the pleura as well. (HE 6.3X)



1-2. Lung, rhesus macaque: Bronchiolar walls are expanded by a combination of smooth muscle hyperplasia, fibrosis, and a prominent cellular infiltrate of macrophages, lymphocytes, and plasma cells, with rare eosinophils. The lumen is ectatic and contains cross section of adult mites. (HE 30X)



1-3. Lung, rhesus macaque: Cross sections of adult Pneumonyssus simicola demonstrate a brown chitinous exoskeleton (black arrows), striated muscle (green arrows), and yolk glands (orange arrows). The exoskeleton of mites of the respiratory tract is thinner than mites living on the skin. (HE 30X)

improved. About a month later a recording was made from the contralateral side of the brain stem. The next day, the animal was reported to be weak with conscious proprioceptive deficits. Over the next few days, the animal deteriorated and developed respiratory distress. Elective euthanasia was performed and lung and brain tissue were subsequently submitted for histologic evaluation.

Gross Pathology: The lungs are multifocally mottled dark red. The brain exhibits dark brown discoloration and cavitation around the 4th ventricle and near the hippocampal formation unilaterally.

Laboratory Results: N/A

Contributor's Histopathologic Description: Lung: In a section of lung, there are variably-sized aggregates of large foamy macrophages, lymphocytes, plasma cells, fewer neutrophils and scattered multinucleated giant cells typically centered on mildly to moderately ectatic bronchioles and less often alveoli and small to medium-caliber vessels. Inflammation infiltrates and expands the walls of affected bronchioles. These bronchioles are lined by severely attenuated to denuded epithelium and multiple coagula of fibrin, foamy macrophages, mature and degenerate neutrophils and multinucleated giant cells. Macrophages and giant cells often contain variable amounts of birefringent golden brown granular intracytoplasmic pigment. Smooth muscle and lymphoid tissue surrounding airways are multifocally and mildly to moderately hyperplastic. Within affected bronchioles and inflammation surrounding alveoli are variable numbers of partial to complete sections of 300-500 µm diameter arthropods characterized by a thin golden chitinous exoskeleton, jointed appendages, a body cavity with striated musculature, and digestive and reproductive tracts (consistent with *Pneumonyssus simicola*). Some of these airways are close to the pleural surface and inflammation extends into and through the pleura, forming a nearly diffuse coagulum of fibrin, foamy macrophages, multinucleate giant cells and few lymphocytes and plasma cells on the pleural surface. The tunicae media and intima of few scattered medium-caliber vessels in the section are diffusely and severely expanded by large foamy macrophages and multinucleated giant cells, narrowing the lumen. The tunicae intima and media of other vessels are obscured by deeply eosinophilic fibrillar material and scattered foamy macrophages and multinucleated giant cells. Other scattered smaller caliber vessels are effaced by aggregates of fibrin, macrophages, lymphocytes and plasma cells. The adventitia of affected vessels is expanded by lymphocytes and fewer plasma cells. In less affected areas of the lung, there is multifocal moderate emphysema and the interstitium is multifocally and mildly expanded by infiltrates of macrophages and lymphocytes. There are scattered areas of mild hemorrhage.

Contributor's Morphologic Diagnosis: 1. Bronchiolitis, alveolitis and pleuritis, necrotizing, histiocytic, lymphoplasmacytic, multifocal, severe, chronic with bronchiolectasis, smooth muscle hyperplasia, lymphoid hyperplasia and intraluminal arthropods (*Pneumonyssus simicola*). 2. Vasculitis, multifocal, necrotizing to histiocytic, severe, chronic with multinucleated giant cells; lung.

Contributor's Comment: While *Pneumonyssus simicola*, the lung mite of macaques, is no longer a routine finding in colony-housed animals, pulmonary acariasis occurs in up to 100% of wild rhesus monkeys and is still seen in research colonies that include wild-caught or imported animals.[1,2,4,6] In all affected macaques, *P. simicola* infection is usually subclinical, but can result in clinical respiratory signs and other complications including pneumothorax and pulmonary arteritis.[4,6] Arteritis characterized by fibrinoid degeneration, macrophages and multinucleate giant cells is present in this case, and the pleural inflammatory reaction and acute onset of respiratory distress suggest an acute pneumothorax was possible prior to euthanasia.

The exact lifecycle has not been fully elucidated, but adult mites are obligate endoparasites and adults feed on host erythrocytes, lymph and epithelial cells in the lung. Transmission requires close association with infected animals as it is likely through direct contact.

Gross lesions are generally multifocal, round, yellow to tan cystic foci up to several millimeters in diameter within the lung parenchyma. Mites occasionally can be visualized in the center of these lesions with the aid of a dissecting scope. Histopathologic findings typically include granulomatous and eosinophilic inflammation centered on the terminal air passages, pigment-laden macrophages, bronchiectasis, alveolar emphysema, bronchiolar smooth muscle hyperplasia and interstitial fibrosis.[1,4-6] Female mites are most commonly seen in the airways and can be up to approximately 700 µm long with a thin chitinous exoskeleton, jointed appendages and a body cavity with a digestive tract and reproductive tract.[3-5] The characteristic birefringent crystalline pigment, regarded as a metabolite of the female mite, can be present in sections of tissue that lack mites and can be used to make a presumptive diagnosis.

While *P. simicola* is most frequently described in rhesus macaques, it has also been reported in other macaque species and a baboon.[3] Pulmonary acariasis is not limited to nonhuman primates; there are numerous species of mites that can be found in nasal passages and lungs of other animals including but not limited to *Rhinophaga* sp. in non-human primates, *Pneumonyssoides caninum* in dogs, *Entonyssus* sp. and *Entophionyssus* sp. in snakes, *Cephenemyia* sp. in wild cervids, *Cytodites nudus* in poultry, and *Sternostoma tracheacolum* in Gouldian finches.

JPC Morphologic Diagnosis: 1. Lung: Bronchiolitis, granulomatous and necrotizing, chronic, multifocal, severe, with bronchiolar smooth muscle hyperplasia, bronchiolectasis and intrabronchiolar arthropods and mite pigment.
2. Pleura: Serositis, granulomatous, multifocal, moderate, with epithelial hyperplasia.
3. Subpleural vasculature, smooth muscle: Hyperplasia, diffuse, mild to moderate.

Conference Comment: This is an exceptional example of pulmonary acariasis with well preserved sections of adults, eggs and often mite fragments within multinucleated giant cells scattered throughout conducting airways and occasionally within alveoli. Bronchiolar walls are often replaced by fibrin and abundant granulomatous inflammation. We observed fibrinoid change in widely scattered vessel in several slides, but it was not constant over the distributed sections, so we have elected not include it in our diagnosis.

The term acariasis equates with a mite infection and is derived from the Order *Acari* in which all mites are classified. While most mite infections are localized to the skin, there are at least ten species of lung mites which infect the lungs of Old World monkeys, all of the genus *Pneumonyssus*.[7]

Conference participants debated on possible causes of the evident granulomatous inflammation with multinucleated cells lining the outside of the pleura in this case. Most agreed with the contributor's suspicions of a secondary pneumothorax, possibly a sequela of ruptured mite houses. Additional Gram, fungal and acid-fast stains did not elucidate any additional infectious organisms. Without definitive causal evidence, we elected to separate out the diagnoses

of serositis and the prominent smooth muscle hyperplasia of subpleural vessels.

As nicely described by the contributor, mite pigment is present in abundance in many sections. This is a golden brown to black pigment usually found within macrophages. This pigment does not contain carbon or melanin, but rather is iron-positive and likely the result of breakdown and excretion of the host's blood proteins.[7]

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CASE II: 13-26805 (JPC 4049055).

Signalment: 6-year-old castrated male Mastiff, dog, *(Canis lupis familiaris)*.

History: Melena and weight loss (duration not indicated). A colonic mass was identified when the patient was placed under general anesthesia and colonoscopy was attempted. The mass was removed per rectum and submitted for histologic examination.

Gross Pathology: Received a 1.5 x 4.0 tan tissue. The surgical border could not be identified at the time of sectioning.

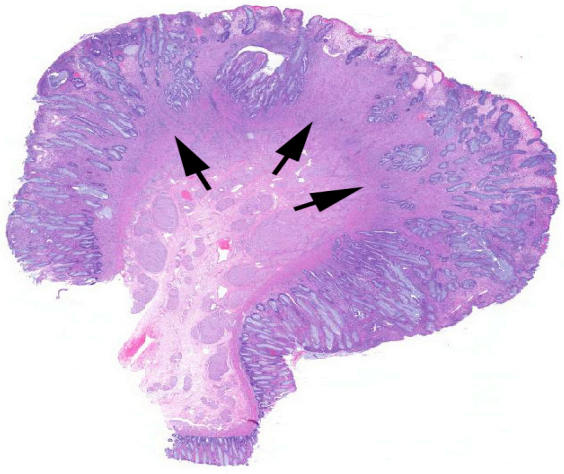
Laboratory Results: The neuronal ganglion cells stained positively with the neuron specific enolase (NSE; see Fig. 1), while Luxol fast blue staining failed to reveal the presence of myelin. Immunohistochemical staining for S-100 and glial fibrillary acidic protein (GFAP) and electron microscopy were not pursued.

Histopathologic Description: The section of the colonic mass is extensively ulcerated with accompanying marked collections of neutrophils, small lymphocytes, plasma cells and hemosiderophages that are also occur as a diffuse infiltrate in the lamina propria. Singleton and small groupings of neuronal ganglion cells are present throughout the lamina propria. The neuronal ganglion cells are polygonal with distinct cell borders and a moderate nuclear to cytoplasmic ratio. The nucleus is eccentric, round to oval with a finely stippled chromatin pattern and a single, prominent, round nucleus. The cytoplasm is moderate and there is a finely stippled to fibrillar pale pink material (Nissl substance) placed eccentrically in the cytoplasm. There are accompanying haphazard to parallel arrays of spindled cells and thin collagen fibers within the lamina propria that extend through the muscularis mucosa, interpreted to be a schwannian stroma. The cell borders are indistinct and the nuclear to cytoplasmic ratio is high. The nucleus is centric, oval to oblong with a finely stippled chromatin pattern and one to three, small nucleoli. The cytoplasm is scant and pink. Mitoses and cellular features of malignancy are not present in the neuronal ganglion cells and schwannian stroma. Profiles of submucosal plexuses are increased in number and size. The muscularis mucosa and serosa are not present in the sections examined.

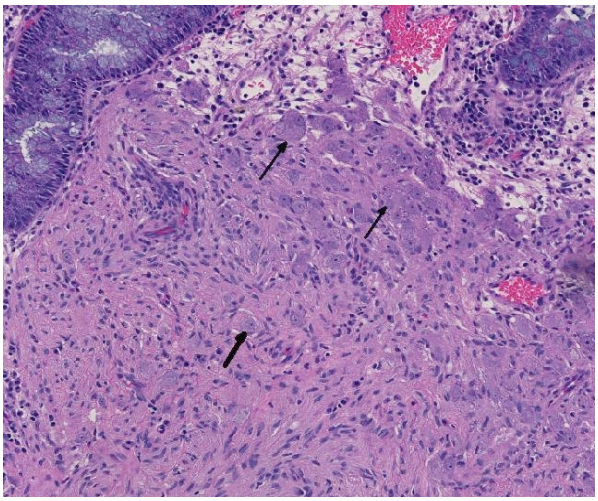
Contributor's Morphologic Diagnosis: Diffuse colonic ganglioneuromatosis with accompanying ulcerative, moderate, mixed inflammatory cell colitis.

Contributor's Comment: The presence of a diffuse infiltrate of ectopic neuronal ganglion cells, a schwannian stroma and hypertrophied and hyperplastic enteric plexuses coupled with the location of the mass warranted a diagnosis of diffuse colonic ganglioneuromatosis (GN) in this dog.

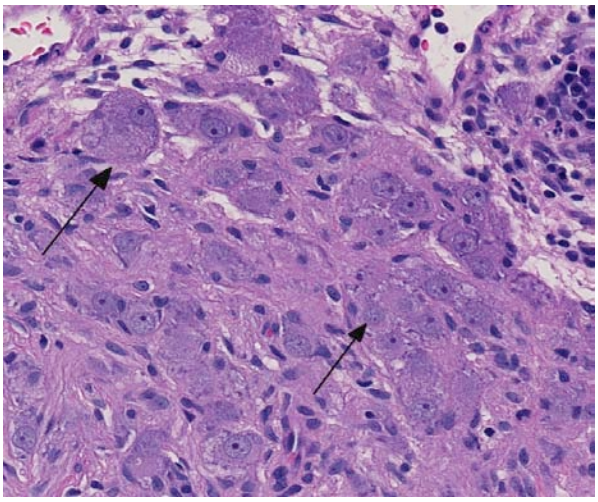
GN is a rare disorder characterized by the abnormal, intramural to transmural, multinodular



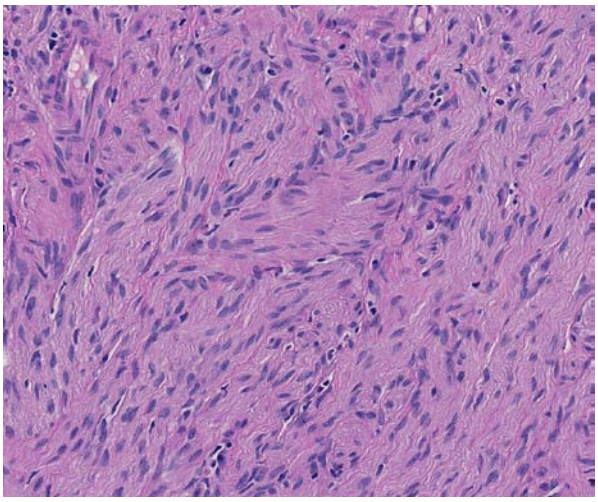
2-1. Colon, dog: A focally extensive area of the mucosa and submucosa is infiltrated by a poorly demarcated neoplasm which separates and replaces mucosal glands (arrows). (HE 6X)



2-2. Colon, dog: The leading edge of the neoplasm contains numerous ganglion cells surrounded by numerous tightly packed spindle cells. (HE 88X)



2-3. Colon, dog: Higher magnification of ganglion cells. (HE 264X)



2-4. Colon, dog: The majority of the neoplasm is composed of tightly interwoven Schwann cells ("Schwannian stroma"). (HE 264X)

to diffuse, proliferation of nerve fibers and ganglion cells in a segment of the intestine.[1,12] The affected segment of bowel is thickened and the lumen can be dilated or narrowed. Human GN can occur anywhere in the gastrointestinal tract but most reported cases involve the colon and rectum.[1,12] Human GN may present as an acute gastrointestinal obstruction or motility disorder or incidentally, during investigations for other gastrointestinal diseases.[1,12] Human hereditary intestinal GN commonly occurs in association with multiple endocrine neoplasia type IIb (MEN-IIb), neurofibromatosis 1 (NF1; von Recklinghausen's disease) and Cowden's disease.[1,4,12] The pathogenesis of human GN remains undetermined. Surgical resection is recommended in human GN when lesions are confined to one section of the intestine.[4] When surgical resection is not an option, symptomatic management is advocated (may include one or more of the following: adjustments, laxatives or enemas, fiber supplementation and gastrointestinal motility modifiers).[4]

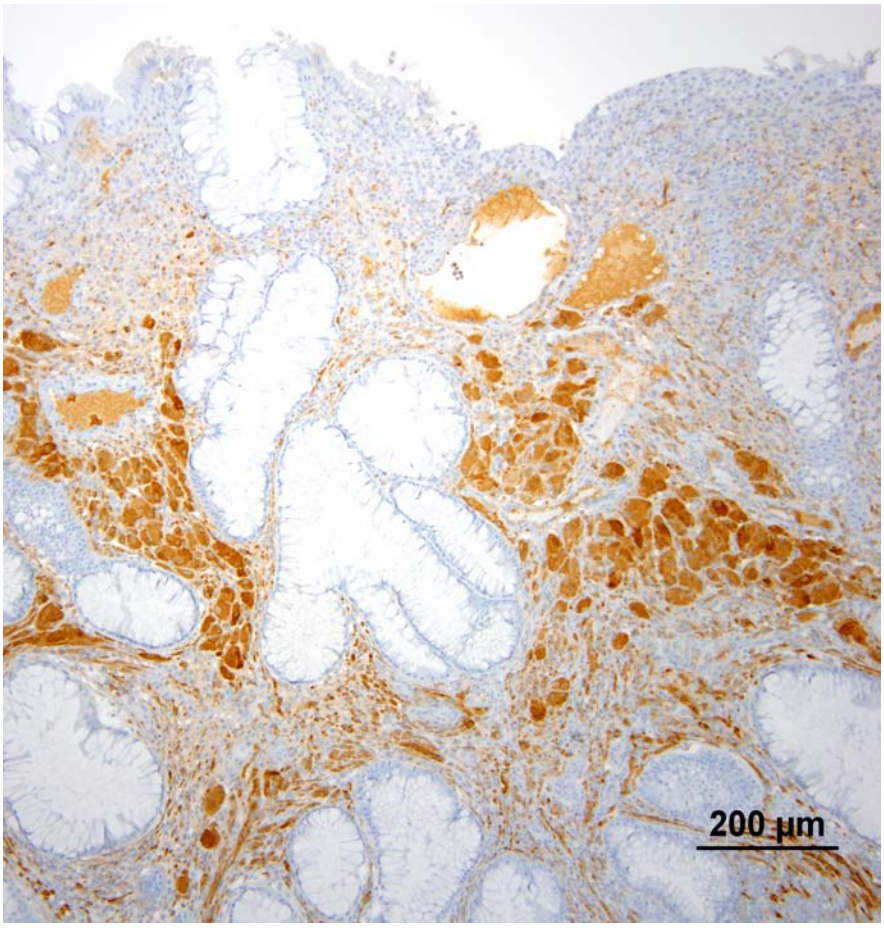
In the veterinary literature, reports of GN have been limited to juvenile and adult dogs, a horse and a steer.[3,5,9,10,12] Affected animals may present with gastrointestinal signs (e.g. vomiting, diarrhea or constipation, hematochezia, melena, tenesmus and abdominal pain) or they can be asymptomatic.[3,5,9,10,12] Abdominal ultrasonography may reveal thickening of the affected segment of the intestine and loss of the normal layers of the intestinal wall.[3,7] Histopathologic examination of full-thickness biopsies from the surgically resected affected segment of the intestine is needed to establish the diagnosis. Immunohistochemistry for neuron specific enolase, S-100 and glial fibrillary acidic protein (GFAP) will aid in establishing the diagnosis.[3,5,9,10,12] There are too few reports in the veterinary literature to comment on the prognosis or behaviour. In two of the reports the affected dogs were euthanized due to the development of a postoperative septic peritonitis.[3,5] There is a single report of a successful outcome following surgical resection in a dog with small intestinal GN.[3]

The pathogenesis of GN in animals is also unknown. It has yet to be established if the genetic mutations that have been identified in human GN occur in animal cases of GN. There is a single report in the veterinary literature in which a duplication of phosphatase and tensin homologue deleted on chromosome 10 (*PTEN)* was demonstrated, using a quantitative multiplex polymerase chain reaction, in a Great Dane puppy with concurrent colorectal hamartomatous polyposis and GN, implying a similar pathogenesis to human Cowden's disease.[3,4,12]

GN should be included as a differential diagnosis in dogs with intestinal thickening and gastrointestinal signs.

JPC Morphologic Diagnosis: 1. Colon: Ganglioneuroma. 2. Colon: Colitis, atrophic and lymphoplasmacytic, chronic, focal, severe.

Conference Comment: Without evidence of multiple sites of origin and given the well-demarcated lesion in sections with adjacent



2-5. Colon, dog: Ganglion cells within the neoplasm are immunopositive for neuron-specific enolase. (anti-NSE, 100X) (Photo courtesy of: Department of Veterinary Pathology, Western College of Veterinary Medicine, 52 Campus Drive, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5B4, Canada. www.usask.ca/wcvm/vetpath)

normal tissue, we prefer the diagnosis of ganglioneuroma in this case. Gastrointestinal pathology specialists at JPC were also consulted and concurred with our diagnosis; however, they commented that the extent of neural tissue within the submucosa is more prominent than is typical in similar cases in people. Although we believe the inflammation and abnormal glandular orientation is secondary to the neoplasm, we elected to separate out these changes in a second diagnosis.

Ganglioneuromas are characterized by exuberant proliferation of all elements of the intestinal ganglia, to include nerve fibers, ganglion cells and supporting cells.[1,7] This is in contrast to less differentiated but related tumors of ganglia origin, neuroblastomas. Neuroblastomas are composed of more primitive-appearing sheets of poorly defined cells with dark nuclei often forming pseudorosettes and lack the more mature ganglion cells of ganglioneuromas. The "schwannian stroma" as nicely described by the contributor and evident with immunohistochemistry for GFAP, organized fascicles of neuritic processes and fibroblasts are all histologic prerequisites for a diagnosis of ganglioneuroma.[8]

Cases may be localized to a mucosal or myenteric plexus, but are more often transmural; this case likely involves both.[1] Conference participants were reminded of the distinct locations within the intestinal wall of Auerbach's plexus (between the inner circular and outer longitudinal layers of muscularis externa) and Meissner's plexus (periphery of the submucosa). They are the sites of synapsis between preganglionic and postganglionic parasympathetic nerves necessary for autonomic control of the intestinal tract.[2]

The association of ganglioneuromas and multiple tumors of all three embryologic layers in Cowden syndrome in people and their correlation with PTEN mutations lend credence to the importance of a normally functioning PI3K/AKT signaling pathway in maintaining homestasis. This tyrosine kinase pathway, initiated by the binding of growth factors, consists of a series of phosphorylation events ultimately resulting in the inhibition of apoptosis (via phosphorylation of BAD, a BCL-2 family sensor & MDM2, direct inhibitor of p53) and enhancement of cell growth and survival (via TSC1/TSC2 inactivation and subsequent mTOR activation). PTEN applies the brakes at the beginning of this cascade where it blocks phosphorylation of PIP_2 into PIP_3 by PI3K.[11] Correlating the PTEN mutation with ganglioneuromatosis in animals has so far been limited to a single report in one dog as previously mentioned by the contributor.[3]

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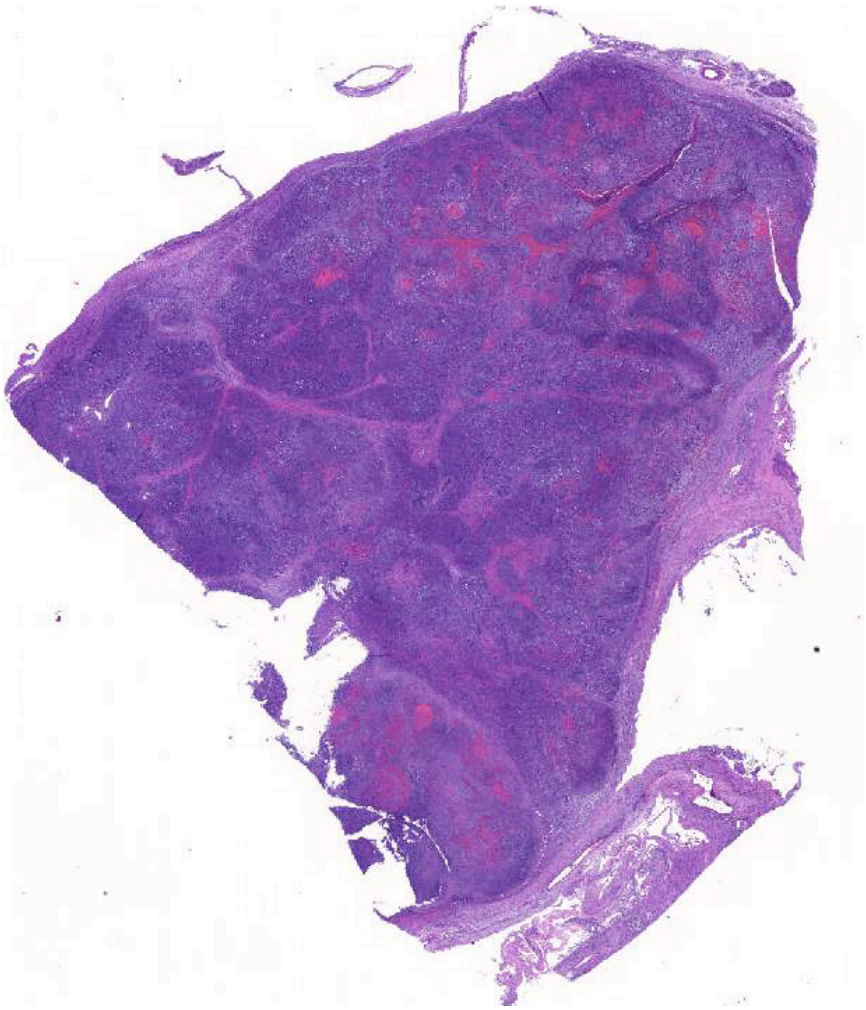
CASE III: 14-19 (JPC 4048513).

Signalment: Adult male rhesus macaque, *(Macaca mulatta)*.

History: This SIV infected adult male Rhesus macaque had a several month history of severe icterus and subsequent terminal malaise, the pathogenesis of which was undetermined. It was sacrificed at its study endpoint and submitted for necropsy evaluation.

Gross Pathology: The pancreas was described as markedly enlarged and firm, with mottled areas of hemorrhage and an accentuated lobular pattern. Some patchy regions of normal appearing parenchyma remained.

Laboratory Results: None



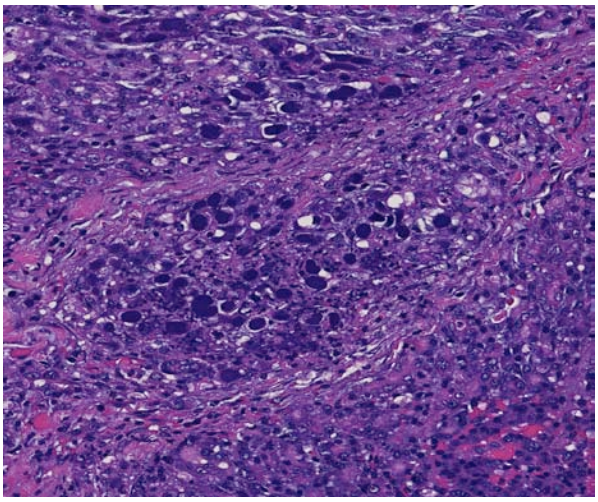
3-1. Pancreas, rhesus macaque: The normal architecture of the pancreas is effaced by diffuse necrosis. (HE 6X)

Histopathologic Description: H&E sections of grossly abnormal pancreas are examined. There is massive, near diffuse necrosis of most lobules, with many demonstrating extensive hemorrhage, severe infiltrates of degenerative neutrophils and prominent acinar cell necrosis, with pyknosis, karyorrhexis and karyolysis. Some sections contain small adjacent and adhered portions of splenic parenchyma, in which there is moderate eosinophilic hyaline amyloid type material centrally within white pulp areas. In many necrotic lobules, remaining identifiable acinar cells contain extremely large basophilic or amphophilic intranuclear inclusion bodies, generally filling and expanding the entire nucleus and sometimes appearing to fuse with cytoplasmic contents, creating nucleocytoplasmic blurring (smudge cells). On highest light microscopy magnification, some of these inclusion structures have a fine interlaced or lattice type pattern visible. Inclusions are noted less frequently in remaining pancreatic ductal epithelium as well.

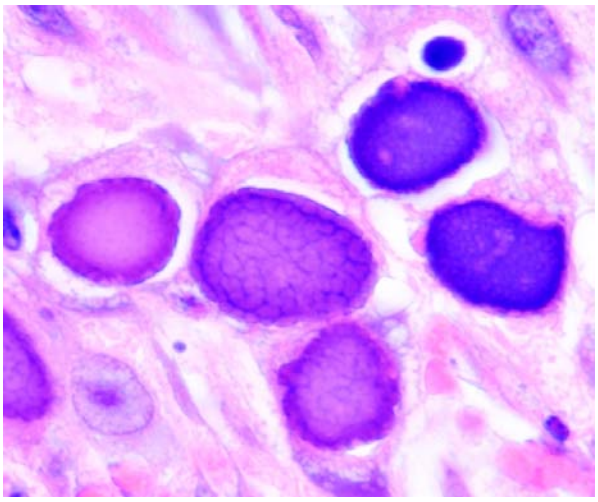
In addition, some lobules not completely necrotic demonstrate prominent regenerative hyperplasia with some atypia. Islet cell structures were infrequently observed and when visible, did not have evidence of primary viral cytopathic effect.

Although the organ was extensively effaced by this necrotizing process, there were small, patchy remaining areas of relatively normal appearing parenchyma (not generally seen in sections distributed). In some sections, overlying pancreatic capsule was markedly thickened and fibrotic, with infrequent fibrous adhesive tags.

Contributor's Morphologic Diagnosis: Pancreatitis, necrotizing and hemorrhagic,



3-2. Pancreas, rhesus macaque: Necrotic pancreatic tissue contains numerous cells with large basophilic intranuclear viral inclusions. (HE 144X)



3-3. Pancreas, rhesus macaque: At high magnification, inclusion structures have a fine interlaced or lattice type pattern visible. (HE 400X)

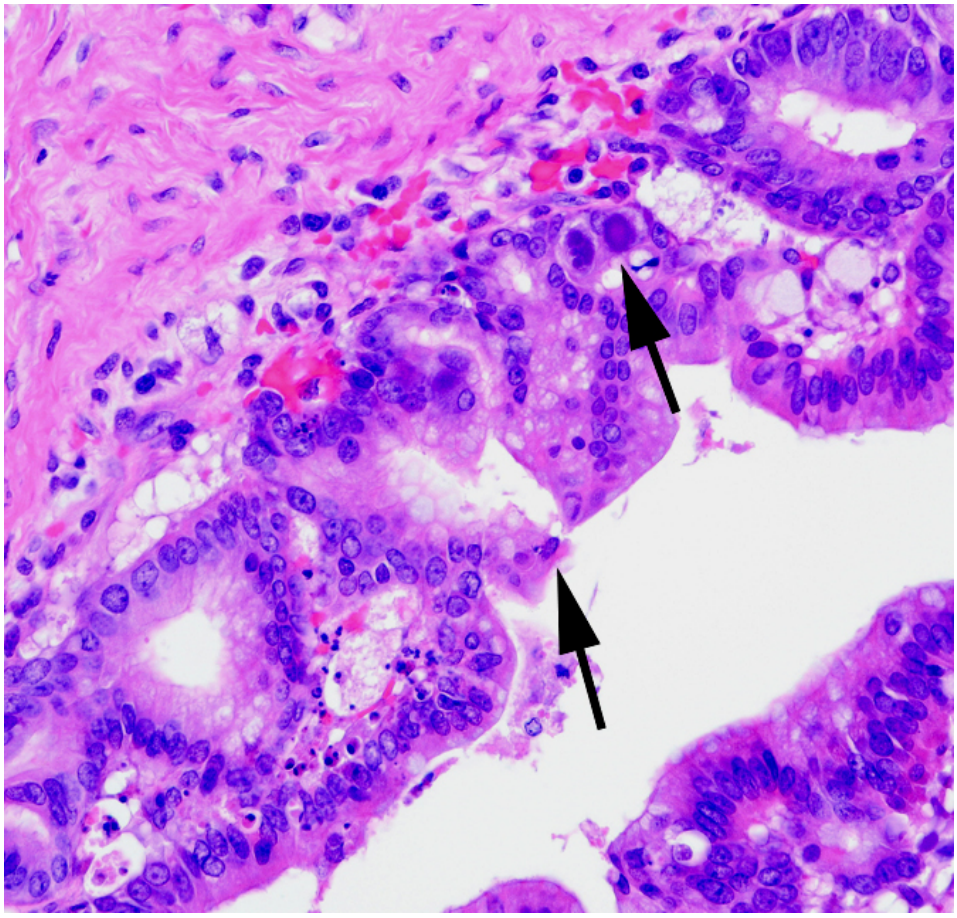
focally extensive to near diffuse, severe, with large basophilic and amphophilic intranuclear inclusion bodies and regions of marked regenerative hyperplasia (some sections).

Contributor's Comment: The microscopic findings are consistent morphologically with the entity of Adenoviral pancreatitis. This spontaneously occurring condition was originally described from a single case in the early 1970's[5] and further reported in the literature as additional individual entities or small clusters on subsequent occasions.[2,3,9,11,12] The paucity of both total cases described as well as absence from retrospective surveys suggests that although this is clearly a defined and consistent entity, it is not a commonly occurring one. An association between retroviral infection and adenoviral pancreatitis has been noted,[6,11] although SIV or other immunosuppressive agents do not appear to be a necessary condition for infection. Most cases are diagnosed based on visualization of characteristic inclusion bodies and the presence of typical adenoviral ultrastructural morphology. In two cases where viral culture has been performed, Adenovirus types 23 and 31 have been isolated.[11,12] Disease has been reported in both juvenile and adult animals, although, unlike this case, the clinical course is typically short, with a rapid (1-2 week) demise, often accompanied by severe bloody diarrhea.[3] Classical signs of acute pancreatitis as described in humans and domestic animal species such as abdominal pain and vomiting are not described. As in previously reported cases of this distinct clinical entity, viral inclusion bodies were not noted outside of the pancreas.[3,5,9,11] However, in several cases of fatal adenoviral pneumonia in non-human primates, intranuclear inclusion bodies were also occasionally observed in bile duct and pancreatic duct epithelium.[10] The presenting jaundiced condition seen in this animal was thought to be due to compression and obstruction of the common bile duct due to pancreatic parenchymal swelling and necrosis. Concurrent hepatic histological findings included prominent canalicular and ductal bile stasis, biliary ductal hyperplasia and mild cholecystitis. Inclusion bodies or other viral cytopathic effects were not noted in hepatocytes or biliary epithelium in this case.

Adenovirus has been isolated from a wide variety of tissues from healthy monkeys[6] and consensus suggests that these viruses usually exist in a latent state, only rarely causing disease,[3] although fatal adenoviral pneumonia has been encountered in a wide range of simian primates.[10] The immunofluorescent-demonstrated presence of duodenal adenovirus antigen was documented in two cases of monkeys with adenoviral pancreatitis and this, along with the common concurrent presence of clinical enteric disease suggests that pancreatic infection may occur from GI ascension through pancreatic ducts.[3] Adenovirus enteritis has been documented in SIV infected Rhesus monkeys.[6]

Necrotizing pancreatitis in animals is not typically associated with an infectious pathogenesis. Foals

have been reported to have naturally occurring adenoviral pancreatitis, although this appears as part of a widespread infection with primary lung and other tissue involvement.[1,3] Experimental pancreatitis has been induced in mice with a wide variety of viruses including Encephalomyocarditis virus (EMC), Reovirus, Coxsackie B virus, Foot and Mouth Disease virus, Venezuelan Equine Encephalomyelitis virus and others.[5,7] Economou & Zissis and others have enumerated infectious causes of acute pancreatitis in humans,[1,4] including multiple viruses such as Mumps, Coxsackie B virus and Hepatitis B virus, although it appears that many of these associations are based on antibody titer presence, clinical presentation and the exclusion of other known causes.



3-4. Pancreas, rhesus macaque: Remaining pancreatic ducts have multifocal areas of necrosis with occasional viral inclusions (arrows). (HE 400X)

JPC Morphologic Diagnosis: Pancreas: Pancreatitis, necrotizing, diffuse, severe, with marked acinar atrophy and loss, and numerous intranuclear viral inclusions.

Conference Comment: In the most common adenoviral diseases of veterinary importance, including the equine adenovirus-1 (intestinal epithelial cells of SCID foals), canine adenovirus-2 (alveolar macrophages of dogs with kennel cough), canine adenovirus-1 (hepatocytes and endothelial cells of unvaccinated puppies), and adenoviral infection of macaques (pancreatic acinar cells of nonhuman primates), the characteristic intranuclear amphophilic inclusions are readily recognized and often associated with prior immune suppression. Adenoviruses occur worldwide and are generally species specific, although transmission between closely-related species can occur, including zoonotic transmission between monkeys and people.[14] Approximately 50 adenoviral serotypes have been identified in nonhuman primates.

Adenoviruses eject DNA from the viral capsid and into the nucleus, where subsequent DNA and protein synthesis create the distinctive inclusions and ultimately result in death of the cell. Ultrastructurally, the inclusions are composed of prominent paracrystalline arrays of virions and unassembled capsid proteins. Respiratory and gastrointestinal epithelial cells are the most common targets of viral replication; however, the epithelial cells of the conjunctiva, cornea, urinary bladder, and kidney, in addition to hepatocytes and pancreatic acinar cells, can also be infected. Although adenoviral inclusions are generally quite distinctive, they can resemble those of cytomegalovirus (herpesvirus) and SV40 (polyomavirus), which also cause significant cytomegaly and nucleomegaly.[14]

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http://pitt.edu/

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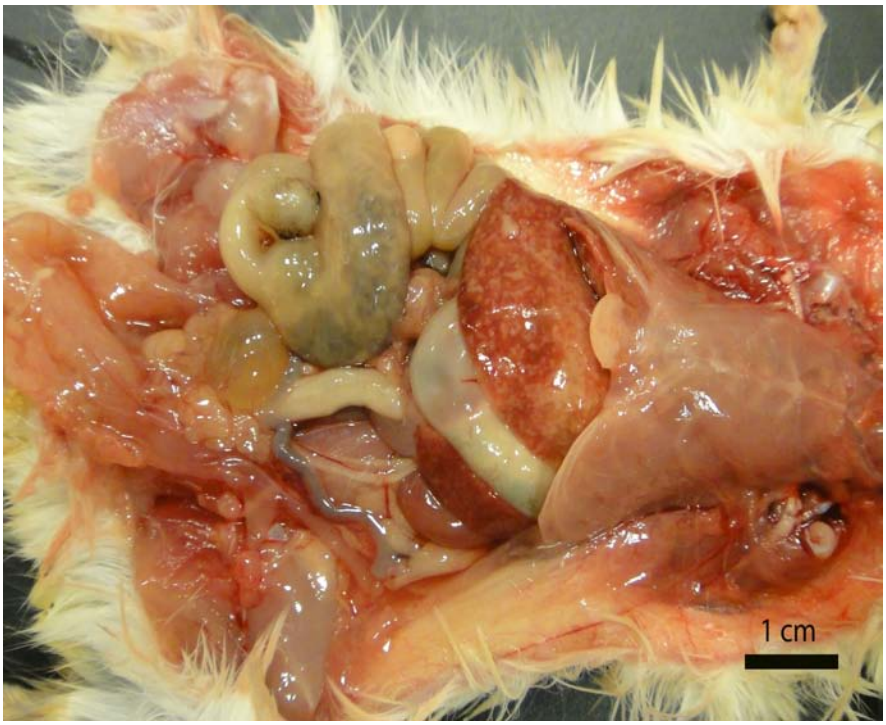
CASE IV: N2013-584 (JPC 4048812).

Signalment: Adult female bushy-tailed jird, *(Sekeetamys calurus)*.

History: A total of 9 bushy-tailed jirds (8 adult and 1 immature; 6 females and 3 males) were found dead within 2 days and without premonitory signs. Three were too autolyzed for further assessment. All the animals came from the same enclosure.

Gross Pathology: Affecting over 80% of the liver parenchyma are coalescing, well demarcated, circular, flat, tan to pale red areas (necrosis). The rest of the parenchyma is mottled red to maroon. Similar foci are scattered throughout the splenic parenchyma. The wall of the large intestine is thickened up to 0.1 cm, pale yellow, has a shiny serosa and contains moderate amounts of green pasty digesta. There are prominent, well demarcated, 0.4 cm in diameter, tan to pale pink lymphoid aggregates that bulge into the serosa. The jird is in fair body condition with small to moderate amounts of subcutaneous and abdominal adipose tissue.

Laboratory Results: Aerobic culture, liver: Many *Listeria* spp.



4-1. Liver, bushy-tailed jird: Multifocal to coalescing areas of necrosis replace 80% of the liver. (Photo courtesy of: Wildlife Conservation Society, Zoological Health Program, Department of Pathology, www.wcs.org)

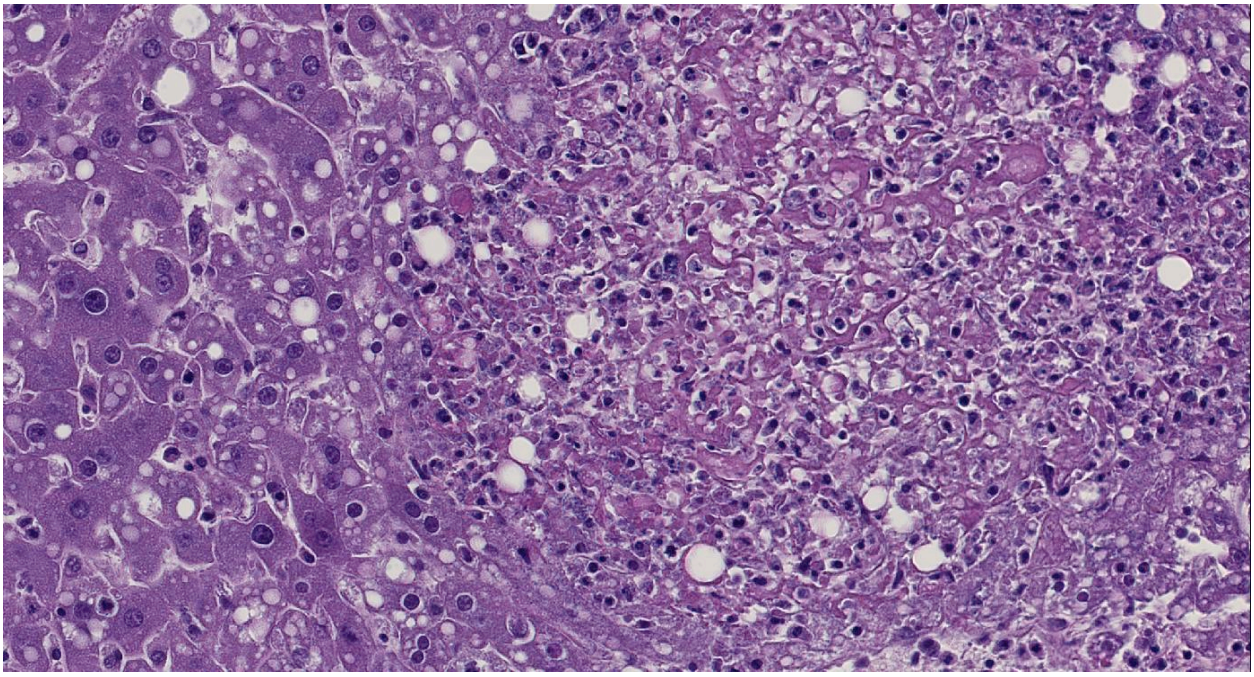
Anaerobic culture, liver: No anaerobes isolated.

Lung or liver from 3 other jirds were sent for aerobic culture and revealed many *Listeria* spp. in two, and many *Listeria monocytogenes* in one.

Histopathologic Description: Liver: Multifocally affecting over 60% of the hepatic parenchyma are randomly scattered, well demarcated, 200 – 500 µm in diameter, coalescing areas of lytic necrosis with large numbers of intralesional, gram positive short bacilli. Bacteria are intra- and extracellular. In most of the sections portal areas are severely affected. The areas of necrosis are characterized by loss of tissue architecture with accumulation of eosinophilic amorphous material, few degenerated neutrophils and cellular and karyorrhectic debris. Necrotic hepatocytes are characterized by shrunken and/or fragmented hypereosinophilic cytoplasm and pyknosis, karyorrhexis or karyolysis. On the periphery of the areas of necrosis hepatocyes often contain multiple 2 – 7 µm in diameter round, clear vacuoles (lipid) and are moderately swollen (degeneration). In blood vessels adjacent or within affected areas there are increased numbers of neutrophils and eosinophilic strand-like material (fibrin) admixed with red blood cells. Multifocally in some of the sections are small areas of sinusoidal and central vein congestion.

Other significant histologic findings (not present in the slide provided) include:

Spleen: Splenitis, necrotizing, acute, multifocal severe with intralesional short bacilli
Lymph nodes, mesenteric and mandibular: Lymphadenitis, necrotizing, acute, diffuse, severe
Intestine: Enterocolitis, necrotizing, acute, multifocal to transmural, severe
Bone marrow: Myelitis, necrotizing, acute, multifocal, moderate
Gram stain: Bacteria are Gram positive.



4-2. Liver, bushy-tailed jird: The liver contains numerous areas of lytic necrosis (right). (HE 400X)

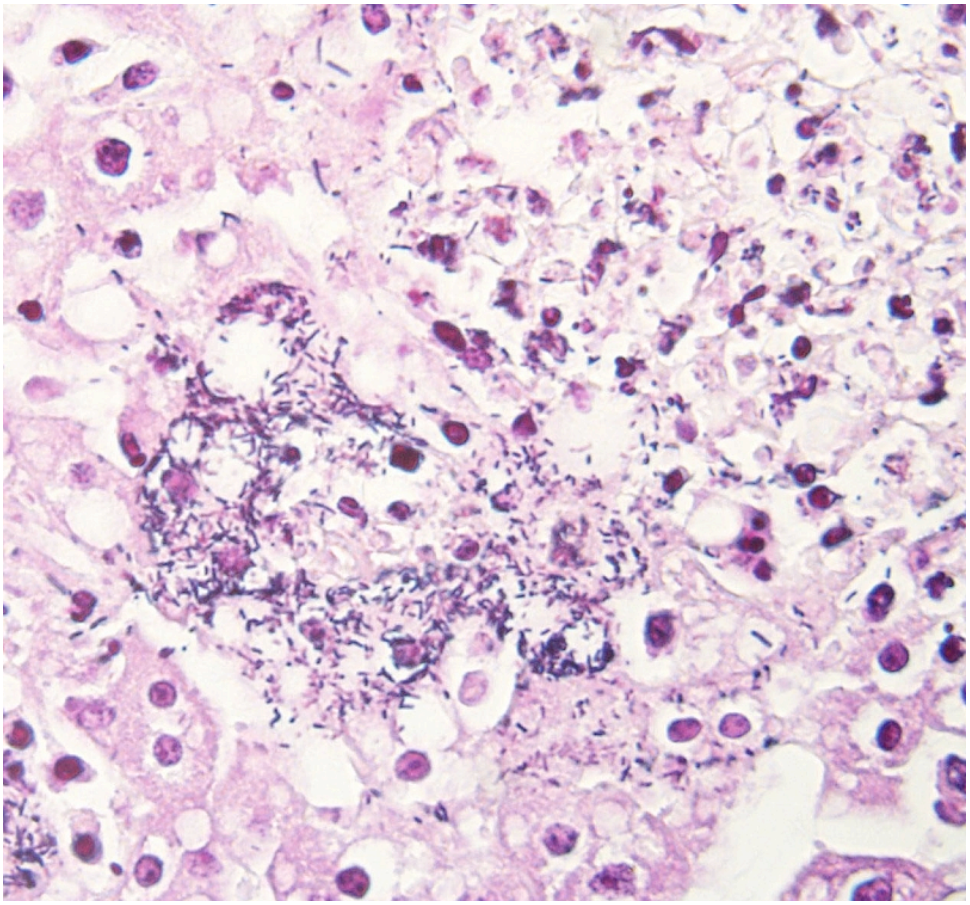
Contributor's Morphologic Diagnosis: Liver: Hepatitis, necrotizing, acute, random, multifocal, severe with intralesional Gram positive short bacilli (*Listeria* spp.).

Contributor's Comment: Gross, histological and microbiological findings in this case are consistent with septicemic listeriosis. Listeriosis is caused by *Listeria monocytogenes* (LM), a Gram-positive, facultative anaerobic bacillus that is ubiquitous in the environment and can multiply in diverse environmental conditions.[8] As an example of LM ubiquity, it can be grown in a temperature range from 4° to 45° C and at a pH range of 5 to 9.[8] This pathogen has been diagnosed in a variety of domestic and non-domestic mammals,[7,10] reptiles[5] and birds.[1,2]

LM has more than 11 serotypes and almost all domestic animal infections are caused by serotypes 1/2a, 1/2b and 4b.[8] It is an intracellular pathogen of macrophages, neutrophils and epithelial cells.[8] Mobile genetic elements in *Listeria* spp. include pathogenicity islands (LIPI-1 and LIPI-2) and plasmids.[6] LIPI-1 encodes for a cluster of virulence factors including hemolysins (such as listeriolysin O)[11,13] and actin-polymerase factors, necessary for the intracellular survival and spread of the organism.[6] LIPI-2 is specific for *Listeria ivanovii* and thought to be responsible for restriction of its host specificity to ruminants and for its lack of typical tropism for the central nervous system observed in LM.[6] Other important virulence factors of LM include the internalins, surface proteins which internalize with E-cadherin to overcome the intestinal, placental and blood-brain barriers.[8]

The three recognized syndromes in domestic animals, that seldom overlap, are infection of the pregnant uterus with abortion, septicemia with miliary visceral abscesses, and encephalitis.[8] In rodents and small mammals the septicemic form predominates.[9] In our jirds, out of the 6 cases in which histology was performed, the organs most commonly affected were liver (6/6), spleen (6/6), large intestine (5/6), small intestine (4/6), lymph nodes (3/4), bone marrow (2/6), lung (1/6) and brain (1/6). Septicemic listeriosis is characterized by multisystemic bacterial colonization with multifocal areas of necrosis or microabscess formation.[8] In our case, the lesions were very acute and only small numbers of neutrophils were recognized within the lesions and adjacent blood vessels. Jirds are gerbil-like rodents[12] that belong to the family *Gerbillinae*. Gerbils are used as models for *Listeria monocytogenes* infection and naturally succumb to the disease.[4]

LM is a zoonotic pathogen that can be transmitted through alimentary sources (food-borne) and direct contact.[11] Food-borne transmission of the



4-3. Liver, bushy-tailed jird: Necrotic areas contain numerous gram-positive bacilli. (Brown and Hopps, 400X)

bacterium was suspected in our jirds, but food was not available for testing. This disease has been previously reported in bushy tailed jirds and the source of infection was similarly not elucidated in that mortality event.[12] Diagnosis was reached by histopathology and microbiology. Gram stains confirmed the presence of gram-positive bacteria in the lesions. Other diagnostic methods that could be of use include immunohistochemistry and PCR.

JPC Morphologic Diagnosis: Liver: Hepatitis, necrotizing, random, multifocal, severe, with numerous bacilli.

Conference Comment: The contributor presents a classic disease entity in its septicemic form and discusses its ubiquitous and hardy nature in addition to describing its molecular interactions with host target cells as highlighted in recent literature. *Listeria* spp. has a unique affinity for the brain stem, and thus is perhaps most commonly associated with its encephalitic form which is almost solely observed in adult ruminants, usually subsequent to consumption of improperly stored silage.[13] Ingestion of the bacteria and its exposure to the host through damaged oral mucosa permits its colonization and ultimate localization to the brainstem via retrograde axonal transport along the trigeminal nerve.[9] It is these cases that present with the typical "circling" behavior and have the characteristic "microabscesses" histologically within the brainstem.

The pathogenicity island LIPI-1 encodes the bacterial surface protein *surface protein actA* which is vital for *Listeria*'s ability to colonize. Once the bacterial population reaches a sufficient size within a single cell, it propels into adjacent cells via actin polymerization of this protein and forms membrane protrusions known as pseudopods. The pseudopods penetrate into adjacent cells forming double-membrane endocytotic phagocytic vesicles which are subsequently lysed by the virulence factor listerolysin-O among others.[13] Transfer of the pseudopod into adjacent cells is facilitated by expression of phosphatidylserine, the well-known surface phospholipid important in both the coagulation cascade and efferocytosis. Effectively, *Listeria* spp. exploits efferocytosis to promote its dissemination. Therefore, a likely target of effective antimicrobial therapy may be phosphatidylserine or its binding receptor for this and other similar bacterial pathogens.[3]

Contributing Institution: Wildlife Conservation Society
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