Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2012-2013

Conference 5

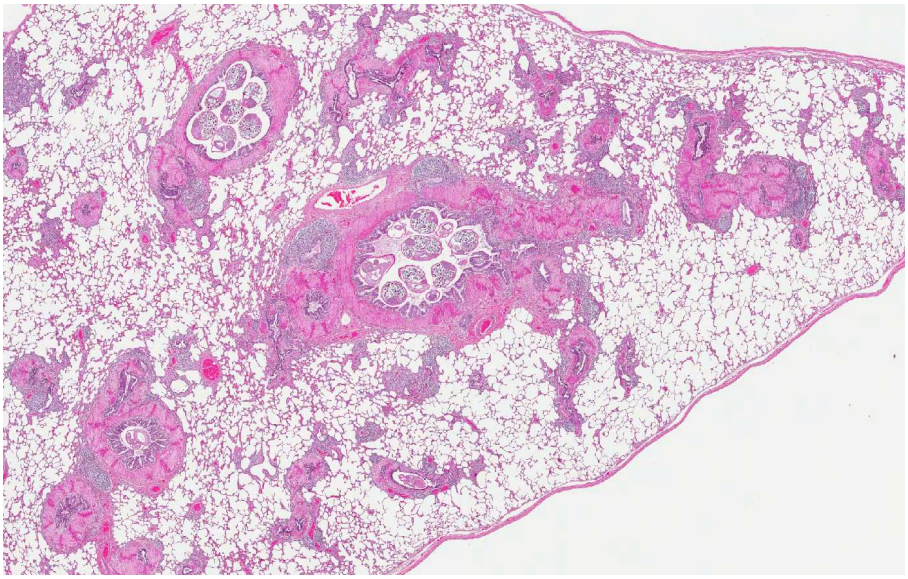
24 October 2012

CASE I: N12-35 (JPC 4018122).

Signalment: 6- to 8-month old, female feral pig, *Sus scrofa,* porcine.

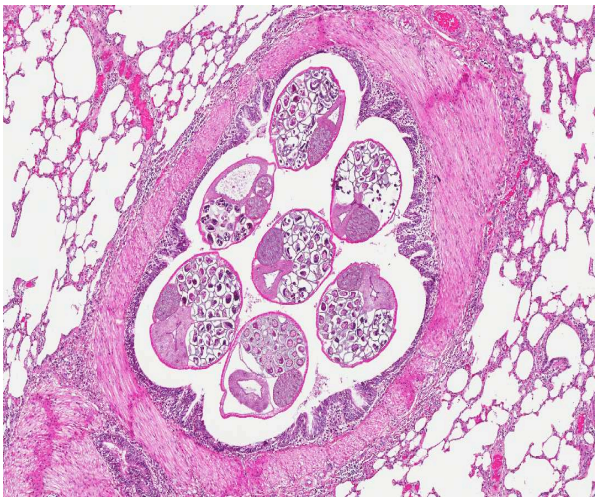
History: This pig was trapped and killed as part of a feral swine monitoring project.

Gross Pathology: A female, black, wild hog in good body condition (BCS 6/9) was presented for necropsy. Numerous live ticks were present on the surface of the body. A 2 cm bullet hole was present caudal to the right eye. Coagulated blood was in the oral cavity. Pink froth was in the lumen of the trachea and extended to the larynx (pulmonary edema). The lungs were diffusely reddened with only approximately 5 cm x 2 cm areas of normal pink lung tissue at the distal poles of the caudal lung lobes. The lung contained large coalescing areas of depressions. The bronchioles of both caudal lung lobes contained numerous white nematodes that were 4-6 cm in length (*Metastrongylus* sp.). The brain contained multifocal areas of hemorrhage (status post brain trauma induced by euthanasia).

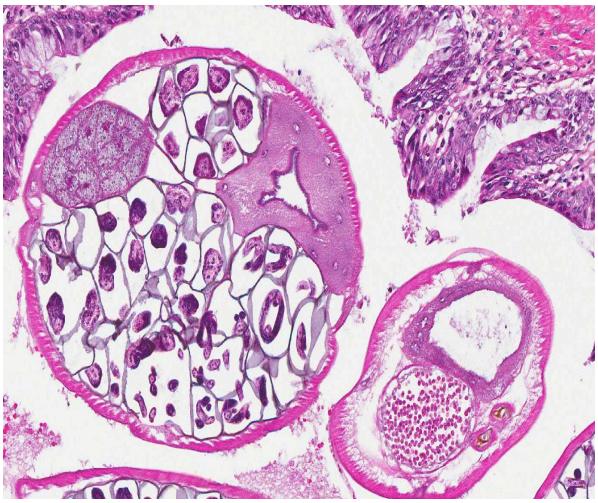


1-1. Lung, pig: Throughout the section, bronchioles are tortuous, exhibit marked smooth muscle and BALT hyperplasia, and contain adult nematodes within lumens. (HE 4X)

Histopathologic Description: Lung. Bronchi and bronchioles contain intraluminal nematodes that are 500-700 micrometers in diameter, with a thin cuticle surrounding a body cavity with coelomyarian musculature, an intestinal tract lined by few multinucleated cells, ovaries, and uteri filled with oocytes



1-2. Lung, pig: Bronchioles contain numerous cross-sections of male (smaller) and female (larger) adult metastrongyle nematodes. (HE 36X)



1-3. Lung, pig: The larger adult females have polymyarian-coelomyarian musculature, a multinucleated intestine (right) and a large uterus filled with larvated eggs. (HE 90X)

and developing larva. The eggs are occasionally free in the lumen of bronchi. There are small to moderate amounts of intraluminal edema, fibrin and mucus admixed with eosinophils and few macrophages, lymphocytes, plasma cells and neutrophils. The bronchial and bronchiolar epithelium is hyperplastic with goblet cell metaplasia and occasionally forms outpouchings. There is marked peribronchial and peribronchiolar smooth muscle hypertrophy. Bronchi and bronchioles are surrounded by moderate to numerous eosinophils with lesser numbers of lymphocytes and plasma cells. There is bronchial associated lymphoid tissue (BALT) hyperplasia. There are multifocal to coalescing areas of alveolar capillary congestion with associated intra-alveolar edema.

Contributor's Morphologic Diagnosis: Lung: Moderate multifocal eosinophilic pneumonia with smooth muscle hypertrophy, goblet cell metaplasia, BALT hyperplasia and numerous intrabronchial and intrabronchiolar nematodes, consistent with *Metastrongylus* sp.

Contributor's Comment: *Metastrongylus* sp. is the common lung nematode that is parasitic in the bronchi and bronchioles of the pig. *M. apri* is the most common, but other species include *M. pudendotectus*, and *M. salmi*.[1] They are nematodes of the superfamily *Metastrongyloidea* and the family *Protostrongylidae*. The intermediate host is the earthworm, and the infective third stage larvae may survive in the earthworm for as long as 18 months. Once the intermediate host is ingested, the larvae migrate through the lymphatics from the intestine to the lungs. Some larvae pass through the liver, producing focal hepatitis, as seen with *Ascaris suum* larval migration. The prepatant period for *Mestastrongylus* sp. is approximately 25 days after which there is a rapid rate of egg production, which eventually subsides to a low level.

Clinical signs include persistent cough, which may be paroxysmal, and growth retardation of the host—which can have a significant negative economic impact in domestic swine.[2] When noticeable, gross lesions typically consist of gray nodules on the pleural surface of the lung, and adult nematodes which can be visualized in bronchi and bronchioles. Heavy pulmonary infections are usually in younger pigs, where worms may be found in all lung lobes. Whereas in older animals, there are typically fewer worms that are often restricted to airways along the caudoventral borders of the caudal lung lobes. Because the earthworm is the intermediate host, pulmonary metastrongylosis is common in wild pigs, but is rare in housed domestic swine. Lungworms are considered to play a role in the transmission of swine influenza.

JPC Diagnosis: Bronchiolitis, eosinophilic and lymphohistiocytic, diffuse, mild, with marked smooth muscle hyperplasia, BALT hyperplasia and numerous cross sections of adult metastrongyles.

Conference Comment: The contributor provided a good summary of metastrongylosis in swine, as well as excellent specimens that allow easy visualization of many of the characteristics of *Metastrongylus* species. In addition to the genus *Metastrongylus*, phylum *Nematoda* contains numerous parasitic species that are of concern in veterinary medicine. When evaluating nematodes in tissue section, it is important to note and describe several key characteristics in a consistent and organized format, paying particular attention to any diagnostically salient features, some of which allow for the diagnosis of a nematode and some of which allow further identification to genus and perhaps even

species. A description of important features of nematodes, and specifically metastrongyles, follows. Nematodes have a cuticle that can vary in thickness from barely discernible to very thick and may be smooth or adorned with bumps, ridges, or wing-like structures. Internal to the cuticle is the hypodermis. In phasmid nematodes such as metastrongyles, lateral extensions of the hypodermis (lateral chords), and dorsal and ventral nerve chords protrude into the pseudocoelom (body cavity), dividing it into quadrants. Additionally, metastrongyles also have accessory hypodermal chords that further subdivide the quadrants. Lateral chords may protrude deeply into the pseudocoelom, or may be low and flat. The hypodermis of phasmid nematodes contains very few nuclei except in the lateral chords. Internal to the hypodermis, nematodes have somatic musculature which either projects into the pseudocoelom in a cylinder-like manner (coelomyarian), or lies low and flat on the hypodermis (platymyarian). Coelomyarian muscle arrangement is such that numerous muscle cells can be present in a single cross section (polymyarian). Conversely, platymyarian muscles are generally larger and fewer (meromyarian). Metastronglyes differ from other members of the strongyle group (i.e. true strongyles and trichostrongyles) by having coelomyarian musculature. Another important feature of nematodes is their digestive tract, which is composed of a mouth, buccal cavity, esophagus, intestine and anus. Esophagus and intestine shape and composition can be useful in identification. Intestines are large, medium or small (described relative to the size of the nematode), and are lined by either many cuboidal to columnar cells, or few multinucleate cells. A brush border of microvilli may be present. Metastrongyles, like other strongyles, have a large intestine lined by few multinucleate cells. Lastly, adult nematodes also have reproductive tracts. In phasmid nematodes, the female has two or more reproductive tracts composed of ovaries, oviducts and uteri. Uteri contain developing eggs or embryos, the presence of which is another identifying feature, as eggs vary among and within groups. Eggs can contain zygotes or larvae; they can be thin- or thick -shelled, or, as in the case of metastrongyle larvae, the shell can be so thin it is not discernible. Metastrongyle larvae have more structure than other nematode larva, which often appear as a sac of nuclei; metastrongyle larvae have a primitive alimentary tract, as well as eccentric tail tips and, in some genera, caudal spines. Adult male nematodes have only one reproductive tract. The testis produces sperm which can be seen in the vas deferens. Sperm can also be found in the female, as the distal part of the uteri (seminal receptacle) stores sperm from previous copulations.[3]

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CASE II: N12-70 RM (JPC 4019377).

Signalment: 11-year-old Thoroughbred mare, *Equus caballus,* equine.

History: The horse was presented with history of coughing, bilateral epistaxis and severe respiratory distress, and later developed colic signs. Thoracic ultrasound examination revealed decreased lung sounds, marked consolidation and pleural effusion on the left side. About 1 liter of thick proteinaceous yellow fluid was removed after placing a chest tube. Cytology of pleural fluid showed few lymphocytes and macrophages. Heart auscultation was within normal limits. Serum chemistry was unremarkable. However, the mare failed to improve despite treatment with broad-spectrum antibiotics and other medications to relieve respiratory discomfort, and was humanely euthanized.

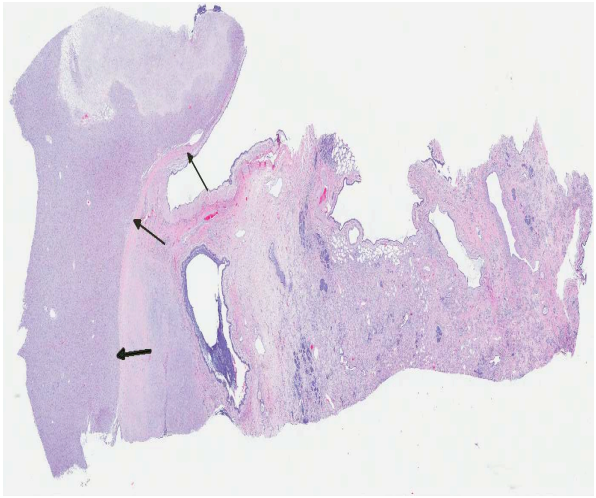
Gross Pathology: The entire left lung is consolidated, firm, and on cut section the architecture is effaced by a multilobulated (0.5-5 cm diameter) tan-pink, firm mass mixed with green-yellow, mucous material. There are 2 liters of serosanguinous pleural effusion in the left hemithorax.

Histological Description: The pulmonary architecture is effaced by a well-demarcated, unencapsulated, expansile, highly cellular mass that surround bronchioles and bronchial glands and compresses the adjacent parenchyma. The mass is composed of sheets of densely packed round to polygonal cells supported by a fine fibrovascular stroma. Neoplastic cells have distinct borders, eccentrically placed nuclei and abundant eosinophilic granular cytoplasm. Nuclei are round to oval and contain finely stippled chromatin and one distinct nucleolus. There are five mitotic figures in ten 400x fields.

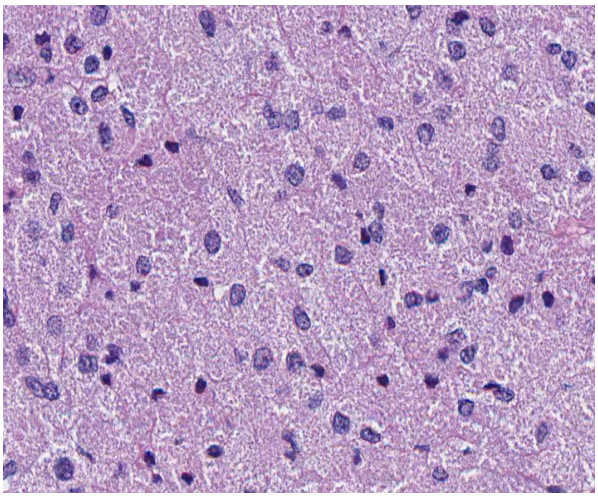
In the adjacent parenchyma, the interstitial septa are expanded (up to 10 times normal) by abundant fibrous connective tissue containing plump fibroblasts mixed with a moderate number of neutrophils, lymphocytes, plasma cells, and macrophages containing intracytoplasmic golden- yellow pigment (hemosiderophages). The bronchoalveolar spaces are filled with a moderate number of degenerate and intact neutrophils, lymphocytes, plasma cells, foamy macrophages, hemosiderophages, necrotic debris, and in few sections they contain granular to fibrillar amphophilic material (mucin). Epithelium lining the bronchioles and alveolar ducts is infiltrated by few neutrophils (exocytosis), and is degenerate (intracytoplasmic vacuolation). Most of the alveolar spaces are compressed by interstitial fibrosis and those remaining are lined by a single layer of low cuboidal epithelium (type II pneumocyte hyperplasia).

Contributor's Morphological Diagnosis: 1. Left lung: Granular cell tumor.
2. Left lung: Marked, diffuse, chronic-active, neutrophilic and lymphohistiocytic bronchointerstitial pneumonia with fibrosis.

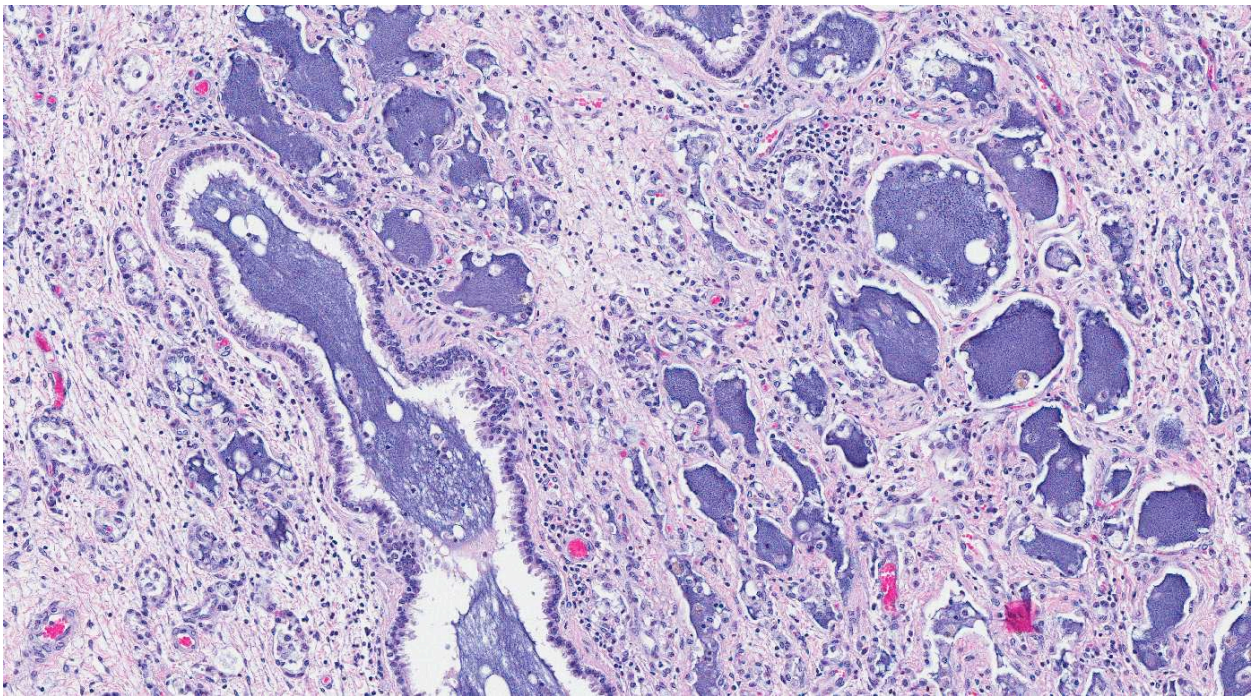
Contributor's Comment: Granular cell tumors (GCT) have been reported in a variety of species such as dogs, cats, rats, horses and humans. They occur on the tongue in dogs; the digits, tongue, and vulva in cats; central nervous system in rats and in a wide variety of sites including subcutaneous tissue in humans.[4,5,7,8] In horses, GCTs arise as primary lung tumors and are more prevalent in aging horses.[4,5] There is no reported sex or breed predisposition. They occur as multiple, peribronchial, white to tan, lobular, well circumscribed, expansile nodules that frequently compress adjacent tissue. They are histologically benign but locally invasive tumors, and are often



2-1. Lung, horse: Compressing adjacent and markedly fibrotic pulmonary parenchyma is an encapsulated, well-demarcated, moderately cellular neoplasm. (arrows) (HE 5X)



2-2. Lung, horse: The neoplasm is composed of densely packed polygonal cells containing numerous, distinct eosinophilic granules. (HE 400X)



2-3. Lung, horse: The adjacent lung is fibrotic with loss of alveolar architecture; remaining airways are often dilated and filled with mucin, and there is mild chronic inflammation. (HE 108X)

recognized as an incidental finding. Ultrastructurally, the neoplastic cells have indented or convoluted nuclei and intracytoplasmic granules that are thought to be accumulations of lysosomes.[6,8]

Although previous reports in humans and in horses strongly support a Schwann cell or neural origin, the histogenesis of GCTs is still controversial.[2,4] While the light and electron microscopic appearance of GCTs in most species is similar, histochemical and immunohistochemical staining varies between the tumor site(s) and species, suggesting multiple cell origins.[2,4] GCTs are vimentin positive and inconsistently positive for S-100, neuron-specific enolase (NSE) and cytokeratin; however, they consistently stain PAS-positive and are diastase resistant. GCTs should be differentiated from other tumors with granular cytoplasm such as oncocytoma (PAS- positive, but sensitive to diastase digestion), rhabdomyoma (desmin- and myoglobin-immunopositive), chemodectoma/carcinoids (packets and nests of cells immunopositive for neuroendocrine markers) and large granular lymphocyte tumors.

In the present case, there was significant involvement of the left lung. Chronic-active pneumonia and fibrosis are secondary to obstruction of the major airways by the neoplasm and the consequential blockage/reduction of normal mucociliary clearance (a critical innate immune response) of pathogens.

JPC Diagnosis: Lung: Granular cell tumor.

Conference Comment: Conference participants discussed several aspects of granular cell tumors (GCTs) adeptly summarized by the contributor, including the occurrence of GCTs in other species, and the controversy regarding their histiogenesis. Human GCTs were once thought to be derived from myoblasts, histiocytes, smooth muscle cells, fibroblasts, or Schwann cells. Immunohistochemical studies suggest that human endobronchial GCTs, the human counterpart of equine pulmonary GCTs, are derived from Schwann cells; as in their human counterparts, the microscopic and immunohistochemical properties of equine pulmonary GCTs indicate that they are of neural crest origin and are most consistent with Schwann cells of the nervous system in the peribronchial/peribronchiolar tissues.[1,3]

In a study of four horses with pulmonary GCTs, all tumors showed uniformly strong positive labeling with antibodies to vimentin, S100 protein, and glial fibrillary acidic protein (GFAP).[3] Vimentin expression is common in cells of mesenchymal origin, and S100 is commonly found in cells of neural crest origin. GFAP is expressed by non-myelin forming Schwann cells in mice and humans. Although myelin-forming Schwann cells express both S100 protein and vimentin, this expression is not specific, as S100 and vimentin antibodies also react with some tumors of non-neural origin. Virtually all tumor cells in all four horses in this study showed positive immunoreactivity to myelin basic protein (MBP) and protein gene product 9·5 (PGP9·5). MBP antibodies react with components of

Schwann cell-derived myelin. PGP9·5 is a major component of neuronal cytoplasm; it is a reliable marker for neurons, and is used in the diagnosis of intraoral granular cell tumors in humans. A few tumor cells from all horses in this study also showed positive immunoreactivity with Leu7. Leu7 reacts with myelin-associated glycoprotein in human neuroectodermal tissue, and some human GCTs have been labeled with antibodies against specific neural markers such as MBP and Leu7. All four tumors lacked expression of neurofilament protein (NF), cytokeratin (CK), chromogranin, α1 antichymotrypsin (AACT), myoglobin, desmin, α-actin and α-SMA, ruling out neuroendocrine or myogenic cell origin. This study, along with others, supports the hypothesis that equine pulmonary GCTs are composed of myelinating and nonmyelinating Schwann cells.[3]

Conference participants uniformly agreed with the contributor's conclusion that the chronic pulmonary pathology in the adjacent lung tissue is secondary to the neoplasm.

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CASE III: S1107769 (JPC 4019380).

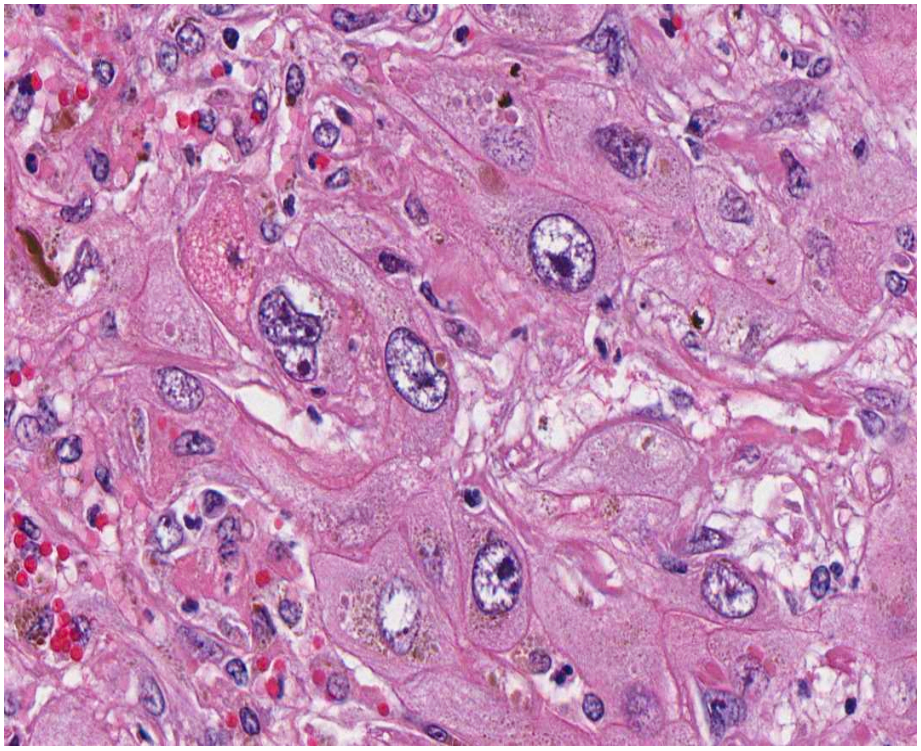
Signalment: 25-year-old Quarter Horse gelding, *Equus caballus,* equine.

History: One of three horses on a ranch in Southern California was found within a week circling and walking aimlessly. According to the submitting veterinarian this horse had "elevated liver enzymes" (specific enzymes and their values were not provided) and it was treated for a week for "hepatic encephalopathy" (no further details provided), after which it was euthanized due to lack of response to treatment.

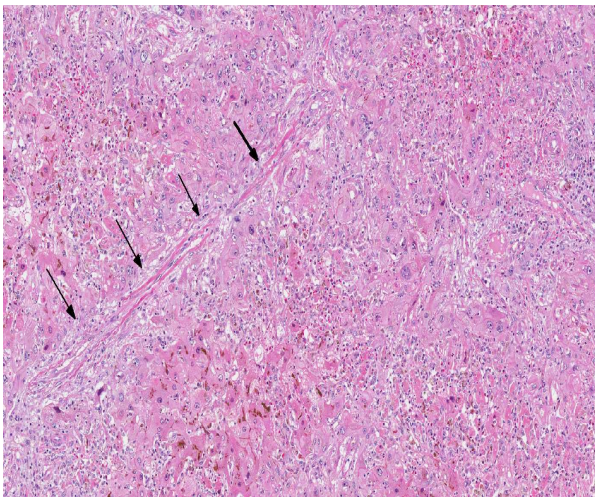
Gross Pathology: The carcass was in poor nutritional condition, with no fat reserves, sunken eyes and mild generalized muscle atrophy. The cardiac and bone marrow (femur) fat showed severe and diffuse serous atrophy. There was a moderate amount (occupying approximately 1/5 of the lumen) of coarse sand, mixed with digesta in all four sections of the large colon. The cecum contained a large amount of reddish, translucent fluid, although no significant gross abnormalities were observed on the mucosa of this organ. The liver was reduced in size (approximately 1/3 to 1/2 of its normal size) and thickness; the consistency was, however, unremarkable. No other significant gross abnormalities were observed in the rest of the carcass.

Laboratory Results: Salmonella culture of liver: negative; Aerobic culture of liver: no growth in 48 hours; Anaerobic culture of colon content: no growth in 48 hours; *C. difficile* culture (colon content): negative; Fecal float: no parasite eggs seen; *C. perfringens* toxins (alpha, beta, epsilon) ELISA in small intestine and colon content: negative; *C. difficile* toxins A/B ELISA in small intestine and colon content: negative; Brain cholinesterase activity: within normal range; Heavy metal screen in liver (including selenium): within normal range. Examination of a bale of alfalfa hay from a batch that this horse and his companions had been eating for the past several months identified the following plants: common groundsel, cheeseweed, wild lettuce, shepherd's purse and green foxtail.

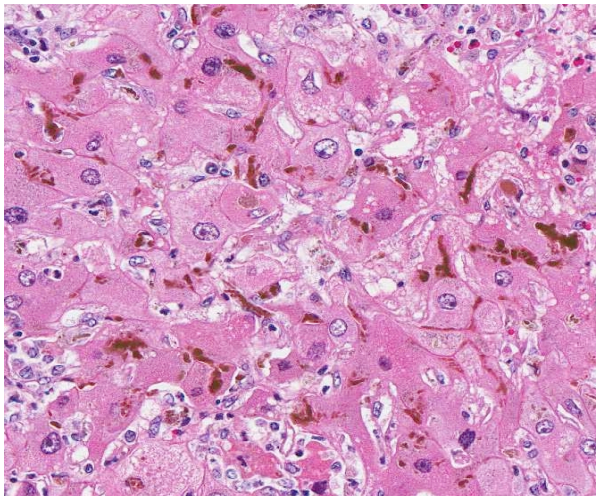
Histopathologic Description: Diffusely, there is severe megalocytosis consisting of enlargement of the nuclei and cytoplasm of a high percentage of hepatocytes. The volume of affected cells is increased as much as to 5 to 10 times. The nuclear membrane is basophilic and sharply delimited. Most hepatocytes have one nucleolus, although up to four nucleoli are present in some cells. The chromatin is fragmented. The peripheral part of the cytoplasm is usually pale and presents multiple coalescing vacuoles. Acidophilic, more or less spherical bodies (cytosegresomes), are occasionally seen in the cytoplasm of some hepatocytes. Because the enlarged hepatocytes are closely apposed, the sinusoids are rarely evident and the general architecture of the organ is distorted. Small to medium size pools of bile (bile stasis) are present in canaliculi and in the cytoplasm of hepatocytes. Multifocally, individual cell necrosis is observed. Diffusely, the liver presents moderate fibrosis, which is mostly restricted to the portal areas, although some fibrosis can be seen attempting to dissect lobules and separate individual cells. Multifocally, there is bile duct proliferation with hypertrophic epithelium. Multifocally there is hemorrhage, and infiltration of neutrophils, lymphocytes, plasma cells and macrophages. Blood vessels in portal areas show



3-1. Liver, horse: There is diffuse distortion of the hepatocellular lobular and plate architecture. Remaining hepatocytes are enlarged up to 2-3X normal and have large, often multiple nuclei and a prominent nucleolus (megalocytosis). (HE 400X)



3-2. Liver, horse: There is marked fibrosis which bridges portal areas (arrows) and occasionally replaces portal hepatocytes (HE 60X)



3-3. Liver, horse: Distortion of hepatic architecture and fibrosis of portal area has resulted in marked cholestasis and numerous bile plugs. (HE 80X)

plump, hypertrophic epithelium. These changes tend to affect all the cells of all the lobules with no special predilection for any part of the lobule.

In the brain (not submitted) there are single or small groups of astrocytes with clear, vesicular nuclei (Alzheimer type II cells), with scant cytoplasm, mostly restricted to basal nuclei, cerebellar peduncles and hippocampus.

Contributor's Morphologic Diagnosis: Hepatopathy, with megalocytosis, portal fibrosis, bile duct proliferation and bile stasis, diffuse, chronic.

Contributor's Comment: The changes described in the liver are classic of pyrrolizidine alkaloids (PA) intoxication, which is consistent with the presence of common groundsel (*Senecio vulgaris*), a plant known to contain PAs, in the hay that this animal was eating.[4] PAs are hepatotoxic, causing irreversible liver damage. Horses and cattle are the major livestock species affected by PAs. Clinical signs of chronic PA poisoning may often not appear for 2-8 months after the first ingestion of PA-containing plants. Affected animals lose condition, and develop liver failure. Neurological signs are commonly seen in horses, and the condition is called "walking disease".[2] After the onset of clinical signs, the prognosis is poor. The most characteristic effect of PA on the liver is the induction of megalocytosis, due to an antimitotic effect. Megalocytosis is not due to inhibition of DNA synthesis, as continued nucleoprotein synthesis, combined with mitotic inhibition, accounts for the increase in size of the nuclei. Although megalocytosis is a hallmark of PA intoxication, this change is not specific for this intoxication, as it can be seen in intoxication by aflatoxins and nitrosamines as well. In this case, however, the hepatic lesions (including megalocytosis, fibrosis and bile duct proliferation) coupled with the detection of *Senecio vulgaris* in the hay that this horse was consuming, is compelling evidence that this horse suffered from PA intoxication.[4] Although PAs can be tested for gastrointestinal contents, the fact that the lesions were so chronic makes testing of doubtful value as it was likely that any PA would have been metabolized long before this animal died. This horse also had neurological clinical signs and presence of Alzheimer type II cells in the brain. These clinical signs and lesions are characteristic of hepatic encephalopathy, a frequent complication of liver failure due to PA intoxication in horses and other animal species. While hepatic encephalopathy in several animal species is characterized by the presence of Alzheimer type II cells and spongiform change of white matter, in the horse, changes are limited to the development of type II cells, which was the case with this animal.[2]

Several toxic plants were identified in the bale of hay belonging to the batch that this animal was eating. Amongst those, the most significant is *Senecio vulgaris* (common groundsel), which was most likely responsible for the hepatic lesions and related clinical signs observed in this animal. *Senecio vulgaris* is a common weed in hayfields in California and is also widely distributed along the East Coast and Canada. Identification of PA-containing weeds in alfalfa and detection of PAs in forage are important to establish an accurate diagnosis.[1] Other plants found in the hay bale are also considered to be toxic, although they probably did not play a role in the disease of this horse. Amongst these, cheeseweed (*Malva parviflora*) is suspected to cause tremors (i.e. shivers or staggers), which may be intensified by exercise, followed by prostration and death. The causative agent is thought to be malvalic and sterculic acid. Wild Lettuce

(*Lactuca virosa*) is reported to cause "opium-like" symptoms. It possesses mild sedative and hypnotic properties. Shepherd's purse (*Capsella bursa-pastoris*) belongs to the mustard family and is reported to cause congenital hypothyroid dysmaturity syndrome in foals. Green foxtail (*Setaria spp.*) is among many species of grass with long, stiff awns or bristles that can cause physical injury to animals. The bristles can puncture sensitive tissue in the mouth and around the nose or eyes. The minutely barbed awns or bristles can work further into a wound by the movement of the animal.[1]

JPC Diagnosis: Liver: Hepatocellular degeneration, necrosis and loss, diffuse, severe, with megalocytosis, bridging portal fibrosis and cholestasis.

Conference Comment: The contributor provides a very good description of clinical and histologic manifestations of pyrrolizidine alkaloid intoxication. There are many PA-containing plants, including species in the *Senecio*, *Crotalaria*, *Cynoglossum*, *Echium, Heliotropium, Amsinckia, Symhytum, Borago, and Trichodesma* genera.[3]

Pyrrolizidine alkaloids are composed of free base and *N*-oxides. Although not directly toxic, once these alkaloids are bioactivated by mixed function oxidases, primarily in centrilobular regions of the liver, the resulting pyrroles are potent electrophiles that bind to and cross-link DNA, proteins, amino acids and glutathione. This results in both cytotoxic and antimitotic effects on hepatocytes. Plant species vary in the amount and variety of PAs they contain; hence the resulting pyrroles vary as well, with some being significantly more hepatotoxic than others. For example, the metabolites of seneciphylline and retrorsine are primarily hepatotoxic, whereas less reactive PAs such as trichodesmine and monocrotaline result in fewer hepatic changes and more extensive extrahepatic lesions. Animal species vary significantly in their susceptibility to toxic effects, likely due to variation in metabolism. For example, the toxic dose of some PA containing plants is 20 times higher for sheep than that for cattle. Relative susceptibility to PA poisoning in domestic animals are: pigs=1 (most sensitive); chickens =5; cattle and horses =14; rats = 50; mice =150; sheep and goats=200 (least sensitive). Additionally, the age, nutrition status and gender of the animal are also important factors in determining the severity of disease. Young animals and animals with suboptimal nutrition are at higher risk, and studies have found male rats are more susceptible than females.[3]

Early cellular changes in PA intoxication are dose-dependent hepatocyte swelling which progresses to degeneration and necrosis. Quickly-ingested high doses result in acute intoxication, characterized by panlobular hepatocellular necrosis, hemorrhage and minimal inflammation. Serum clinicopathologic abnormalities in acute toxicity include elevations in aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), alkaline phosphatase (ALK), and gamma-glutamyl transpeptidase (GGT) as well as increased bilirubin and bile acids. When lower doses are ingested over a longer period of time, chronic poisoning results. Early chronic lesions include piecemeal hepatic necrosis, minimal peribiliary fibrosis and mild bile duct regeneration. With time, megalocytosis and fibrosis occurs, as in this case, and animals eventually develop clinical liver failure and sequalae such as photosensitization, icterus, and increased susceptibility to hepatic lipidosis and ketosis. In chronic cases, animals may develop transient elevations of serum enzymes, bilirubin and bile acids; however, these may remain within normal limits for months after the PA ingestion.[3]

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CASE IV: 110592-22 (JPC 4019408).

Signalment: Aged male common marmoset, *Callithrix jacchus,* non-human primate.

History: This colony marmoset was housed at the U.S. Army Medical Research Institute of Infectious Diseases. The marmoset was euthanized because of chronic weight loss and lethargy.

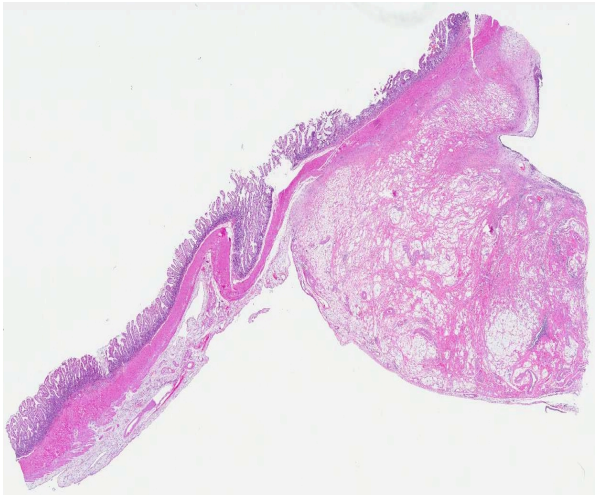
Gross Pathology: The wall of the proximal jejunum was circumferentially thickened and firm for a length of approximately 0.5 cm with marked narrowing of the lumen ("napkin-ring" appearance/stricture). Other necropsy findings included: minimal subcutaneous and visceral adipose tissue; several missing and worn incisor teeth; mild splenomegaly; a moderately enlarged, pale tan liver with patchy white to pale yellow coalescing areas that bulged on cut surface; and few, approximately 0.5 mm diameter, white, slightly raised foci on the capsular surface of the right liver lobe.

Histopathologic Description: Jejunum: Expanding and infiltrating the mucosa, submucosa, and tunica muscularis is an unencapsulated, poorly circumscribed, moderately cellular neoplasm composed of polygonal cells (epithelial origin) arranged in disorganized acini and nests often separated and surrounded by an abundant amphophilic finely fibrillar material (mucin). Neoplastic cells have generally distinct cell borders, small to moderate amount of eosinophilic cytoplasm, round nuclei with finely stippled chromatin, and 1-2 distinct, occasionally prominent, nucleoli. A subpopulation of neoplastic cells is rounded with amphophilic cytoplasm that peripheralizes the nucleus (signet-ring appearance). There is moderate anisokaryosis with occasional multinucleate neoplastic cells. Mitotic figures are not observed. The tunica muscularis is markedly expanded (up to 5x normal) by fibrosis and reactive fibrous tissue (desmoplasia). There are moderate numbers of lymphocytes and plasma cells with fewer macrophages, neutrophils, and eosinophils scattered within the neoplasm. Multifocally, moderately expanding the basement membrane of vessels and adjacent connective tissue within the lamina propria is a pale eosinophilic amorphous material (consistent with amyloid). Other findings in some sections include: villous fusion and blunting (atrophy); erosion/ulceration; crypt abscesses; serositis with reactive mesothelium; and lymphoid follicular aggregates in the deep tunica muscularis.

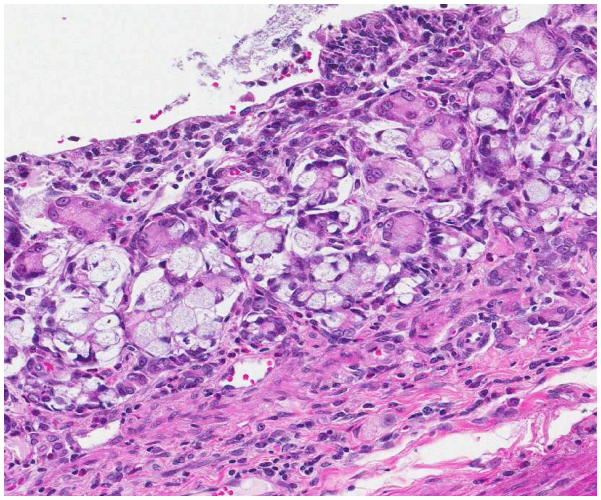
Contributor's Morphologic Diagnosis: 1. Jejunum: Adenocarcinoma, mucinous. 2. Jejunum, lamina propria: Amyloidosis, multifocal, mild.

Contributor's Comment: Small intestinal adenocarcinomas are generally uncommon in human and nonhuman primates. The most commonly reported intestinal carcinomas in nonhuman primates are ileocecal adenocarcinomas in aged rhesus macaques (*Macaca mulatta*) and colorectal adenocarcinoma in cotton-top tamarins (*Saguinus oedipus*).[5] In humans, most small intestinal malignant tumors are metastases from tumors arising in other locations with colorectal adenocarcinomas much more commonly reported.[1] The association of chronic-active colitis with development of colorectal adenocarcinomas in cotton-top tamarins and humans is also generally well-accepted.[3,5,6,8]

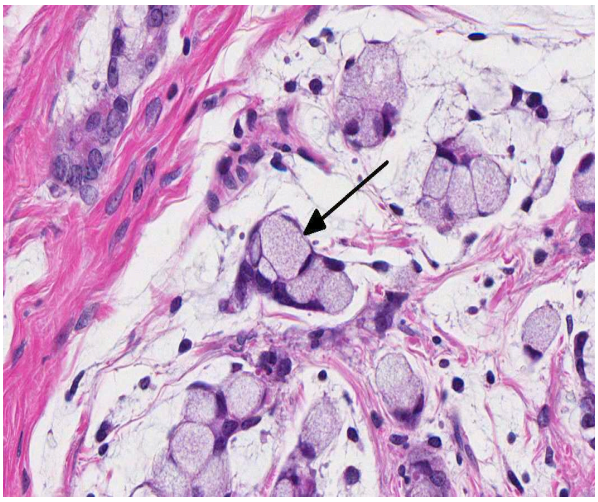
A recent report suggests that small intestinal adenocarcinoma may be a relatively common neoplasm in aged common marmosets (*Callithrix jacchus*).[5] In this report, tumors were usually located



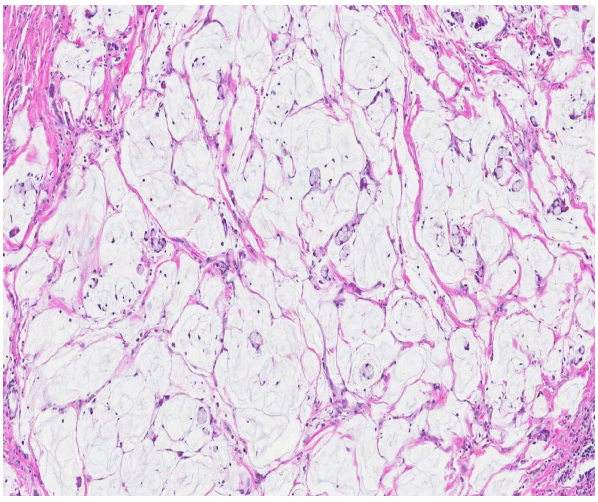
4-1. Small intestine, marmoset: There is transmural infiltration and marked expansion, especially of the serosa, by an unencapsulated, poorly demarcated neoplasm. (HE 5X)



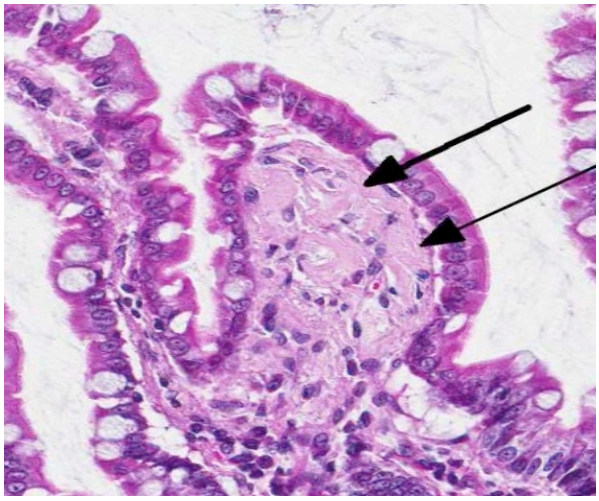
4-2. Small intestine, marmoset: Neoplastic cells infiltrate the mucosa resulting in architectural distortion as well as marked blunting of overlying villi. (HE 80X)



4-3. Small intestine marmoset: The large "lakes" of mucin surrounding neoplastic cells is a characteristic feature of the mucinous type of intestinal adenocarcinoma. (HE 92X)



4-4. Small intestine, marmoset: Nests of neoplastic cells are often surrounded by lakes of clear to amphophilic mucinous matrix. In some neoplastic cells, large vacuoles of amphophilic mucin peripheralize the nucleus (signet ring cells)(arrow). (HE 400X)



4-5. Small intestine, marmoset: Multifocally, the adjacent lamina propria is multifocally expanded by a waxy, eosinophilic homogeneous material (amyloid). (HE 200X)

within the proximal small intestine, immediately distal to the duodenum, with grossly observed thickening and stricture at the tumor site often present. Signet-ring cell differentiation, lymphatic infiltration, and metastatic spread to the regional lymph nodes were other common findings. Carcinomatosis was not observed. An association between presence of callitrichine herpesvirus 3 (marmoset lymphocryptovirus) or *Helicobacter* sp. and tumor development was not found.[5] This case shares similar features to those reported; however, evidence of metastasis was not observed in histologically examined tissues.

The reason for the predisposition of development of small intestinal adenocarcinoma at the duodenal-jejunal interface is unknown; however, there is a belief among some pathologists that components of biliary or pancreatic secretions that enter the intestine at this location may result in cell damage and subsequent tumorigenesis.[1] Even though the small intestine has a high cell turnover and large epithelial surface, adenocarcinomas develop much less frequently than in the large intestine. Several hypotheses have been postulated to explain this disparity in occurrence and these include: dilution of potential carcinogens due to the more liquid nature of small intestinal contents allows decreased mucosal contact; rapid transit time in the small intestine allows decreased mucosal contact of potential luminal carcinogens; presence of Paneth cells in the small intestine aids in antibacterial activity; lack of anaerobic bacteria in the small intestine that are able to convert bile salts to potential carcinogens; large amounts of lymphoid tissue (lamina propria and Peyer's patches) that provide increased immunosurveillance against tumor cells; large amounts of mucosal enzymes that can detoxify luminal contents; less mechanical trauma to the mucosa due to more liquid luminal contents; and crypt stem cells are further away from contact with potential luminal carcinogens.[1]

Intestinal adenocarcinomas can often be further described based on their predominant morphologic appearance into acinar, papillary, mucinous, signet-ring cell, undifferentiated, or adenosquamous subtypes. In general, small intestinal adenocarcinomas are uncommon in domestic animals. However, in some countries, small intestinal adenocarcinoma can be common in cattle and is associated with ingestion of bracken fern and bovine papillomavirus type 4 infection.[2] Tumors are usually multiple and vary from adenoma to carcinoma affecting all levels of the small intestine. In sheep, there has also been an association

with bracken fern ingestion and herbicide use.[2] Unlike in cattle, these tumors are usually mid-jejunal and solitary.

An additional finding in this marmoset was the presence of a pale eosinophilic amorphous material in widespread tissues (gastrointestinal tract, spleen, liver, kidney, adrenal gland, and thyroid gland). Congo red stain of a replicate section of kidney from this marmoset confirmed that the eosinophilic material is amyloid. Systemic AA or reactive secondary amyloidosis has been reported in common marmosets, usually related to a chronic inflammatory process that results in elevated serum amyloid A (SAA) protein levels. A genetic factor may also play a role in the common marmoset.[4] In addition to the small intestinal adenocarcinoma, this marmoset exhibited marked to severe granulomatous and eosinophilic cholangitis with intralesional degenerate parasitic remnants suggesting that this chronic inflammatory process likely contributed to elevated SAA levels with resulting widespread amyloid deposition.

Note: Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Research involving this marmoset was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011. This research was conducted under an Institutional Animal Care and Use Committee (IACUC) approved protocol. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

JPC Diagnosis: 1. Jejunum: Adenocarcinoma, mucinous type.
2. Jejunum, lamina propria: Amyloidosis, multifocal, moderate.
3. Jejunum: Ulcer, focal.

Conference Comment: The contributor provides an excellent discussion of intestinal adenocarcinomas in non-human primates, as well as the association between inflammation, cell damage and tumorigenesis. Conference participants discussed the pathogenesis of colorectal cancer in humans and nonhuman primates, noting that similar pathogeneses are proposed for both colorectal and small intestinal adenocarcinomas.[5] Colorectal adenocarcinomas account for 15% of all human cancer-related deaths in the United States, making it the second leading cause of cancer-related deaths, behind lung cancer.[7] While numerous infectious, genetic and environmental factors contribute to the development of gastrointestinal tumors, two distinct genetic pathways have been implicated with playing major roles in the development of intestinal adenocarcinomas: the Wnt signaling pathway (involving APC and β-catenin) and the microsatellite instability pathway.[5,7] Wnt is a signaling pathway that regulates β-catenin. The APC (adenomatous polyposis coli gene) is a potent tumor suppressor that, under normal conditions, complexes with and phosphorylates β-catenin, marking it for ubiquitination and ultimately destruction. When Wnt is activated, the destruction complex between APC and β-catenin is deactivated, resulting in increased cytoplasmic β-catenin levels. As levels rise, β-catenin translocates into the nucleus, where it binds to TCF, a transcription factor that activates genes such as MYC and cyclin D1 that promote proliferation and increase cell cycle progression. Loss or mutation of APC can likewise lead to increased levels of β-catenin and cell cycle promotion and growth. This is followed by other mutations, such as activating mutations in KRAS, SMAD2 and SMAD4, which further promote cell proliferation and inhibit apoptosis. SMAD2 and SMAD4 are effectors of TGF-β signaling. TGF-β normally inhibits the cell cycle; hence, loss of these genes can allow unregulated cell proliferation. Additionally, mutations (chromosome deletions) in the tumor suppressor gene p53 also occur in later stages of tumor progression. Mutations such as these are due to chromosomal instability, which is a hallmark of APC/β-catenin pathway.[7] Another pathway implicated in the development of intestinal adenocarcinomas is microsatellite instability, which occurs when DNA mismatch repair is deficient, allowing mutations to accumulate in microsatellite repeats. Generally, microsatellites are in noncoding regions, but some microsatellite sequences are located in coding or promoter regions in genes that regulate cell growth (i.e. genes encoding type II TGF-β receptor and the proapoptotic protein Bax). Type II TGF-β mutations can contribute to uncontrolled cell growth, whereas loss of Bax may enhance the survival of neoplastic cells.[5,7]

In addition to playing a role in the Wnt signaling pathway, β-catenin activity also has effects on cell-cell organization. β-catenin normally binds to the cytoplasmic domain of type I cadherins, facilitating linkage to the actin cytoskeleton and contributing to normal cellular structure and organization. Perturbed interactions between β-catenin and type I cadherins destabilize cell-cell interactions and thus promote the loss of cell cohesion which may contribute to metastatic spread of neoplastic cells.[7]

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