The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 24

7 May 2008

Moderator:

Dr. Thomas P. Lipscomb, DVM, Diplomate ACVP

CASE I – C-33738-06 (AFIP 3083594).

Signalment: Adult, spayed, female Chihuahua

History: This dog developed vomiting, diarrhea and anorexia one day following routine vaccinations and was treated symptomatically with fluids and antibiotics. However, the patient continued to deteriorate with evidence of progressively worsening liver disorder and was euthanized eight days following the vaccinations.

Gross Pathology: The submitting veterinarian did a necropsy on the patient but did not report abnormal findings. Fixed specimens of spleen, kidney and liver were received from the referring veterinarian for histopathology.

Histopathologic Description: The section of liver is characterized by marked centrilobular and midzonal hepatic necrosis **(Fig. 1-1)** with sparing of hepatocytes located adjacent to portal triads. Canalicular plugging with bile is frequently observed between surviving hepatocytes **(Fig. 1-2)**. In the necrotic tissue, ghost-like remnants of necrotic hepatocytes and the accompanying sinusoids can generally be visualized (coagulative necrosis). Inflammatory cell activity is minimal in all areas.

Contributor's Morphologic Diagnosis: Marked acute hepatic necrosis with periportal sparing and periportal intrahepatic cholestasis, Chihuahua, canine.

Contributor's Comment: Upon further investigation, the referring veterinarian discovered that the patient had inadvertently been vaccinated by injection with an intranasal trivalent *Bordetella bronchiseptica*–canine parainfluenza-canine adenovirus-2 vaccine product due to an error in vaccine preparation by a newly hired technician. The product package insert warns that subcutaneous or intramuscular administration of the intranasal product may result in icterus or death from liver failure, but we were unable to find any information in the scientific literature to explain the mechanism of hepatic injury or which component in the vaccine might be responsible for the injury. There is one published report of acute hepatic necrosis associated with subcutaneous administration of an intranasal canine Bordetella-canine parainfluenza vaccine, but the authors did not speculate as to pathogenesis. The patient survived and hepatocellular disease was still present two months later based on hepatic biopsy and serum bile acid concentrations.[5] Equine serum hepatitis, sometimes known as Theiler's disease, occurs subsequent to vaccination with biologics that contain equine serum and has a similar pattern of marked hepatic necrosis with periportal sparing. However, after nearly one hundred years since equine serum hepatitis was first reported, the pathogenesis of the disorder remains elu-

sive.[2] Ordinarily, massive hepatic necrosis in dogs suggests a toxic etiology. Although many drugs, toxins and chemicals have been shown to cause hepatic injury in dogs[2], it is difficult to find a comprehensive list of substances in which the toxicosis in dogs is predominately manifested by acute, severe hepatic necrosis. In our laboratory, ingestion of xylitol, cycad palm, poisonous mushrooms (particularly *Amanita* sp.), or water containing blue-green algae are our first considerations as causes of marked hepatic necrosis when there has been no known exposure to drugs or chemicals.

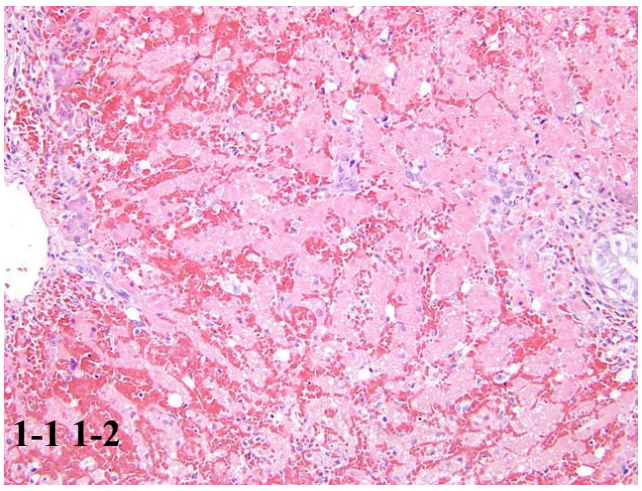
AFIP Diagnosis: Liver: Hepatocellular necrosis, acute, submassive to massive, diffuse, with hemorrhage and canalicular cholestasis, Chihuahua (*Canis familiaris*), canine.

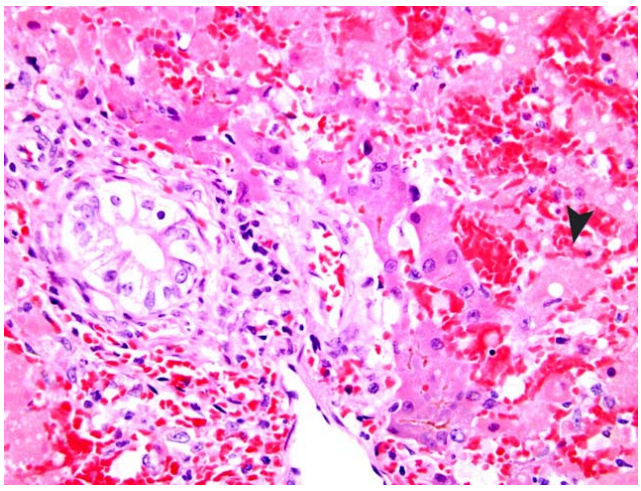
Conference Comment: Massive hepatic necrosis is defined as necrosis of entire acini. In the sections examined at the conference, there are acini that are entirely necrotic as well as acini that are largely necrotic with a rim of surviving hepatocytes around the portal areas. Massive necrosis leads to collapse of the remaining stroma, impaired regeneration and postnecrotic scarring. It is usually, but not always, caused by toxins. Hepatosis dietetica is a nutritionally induced form of massive hepatic necrosis. [4]

Hepatotoxic agents can be divided into two broad categories based on their predicted activity. Predictable hepatotoxins are those that produce a generally consistent activity in the majority of the animals that are exposed. The extent of injury produced in an individual animal by a predictable hepatotoxin may differ depending on various factors including age, sex, diet, and endocrine function. Idiosyncratic drug reactions are caused by those agents that produce an effect in a small minority of the animals exposed, such as carprofen occasionally causing acute hepatic necrosis in Labrador retrievers and diazepam causing acute fatal hepatic injury in some, but not all, cats.[2]

Hepatotoxic agents can be classified into six different categories based on their cellular target.[2]

1. Production of toxic metabolites by the cytochrome p450 system is the most common form of hepatocellular injury. The enzymes of this system are located in the smooth endoplasmic reticulum and are found in the highest concentration of centrilobular hepatocytes. They function to metabolize lipid-soluble chemicals into water-soluble compounds for excretion.
2. Drugs and cellular enzymes may combine together to form neoantigens. When transported to the cell surface and presented as antigens, these neoantigens may stimulate both cellular and humoral immune responses resulting in either direct cellular cytotoxicity or antibody-dependent cellular cytotoxicity. (halothane)
3. Some toxins may directly initiate apoptosis by stimulating proapoptotic pathways within hepatocytes. (hydrophobic bile acids)
4. Certain toxins may directly damage cellular membranes disabling calcium homeostasis and resulting in cell death. (carbon tetrachloride)
5. There are chemicals that will bind and disrupt the ca-





1-1. Liver, Chihuahua. Diffuse coagulative necrosis of the hepatic cords. (HE 40X).
1-2. Liver, Chihuahua. Multifocally, hepatocytes of the limiting plate are often degenerate characterized by swollen, pale, vacuolated cytoplasm (arrowhead) and/or contain green-brown intracanilicular bile plugs (cholestasis). (HE 400X).

Selected hepatotoxins extracted from Cullen[2]

Category	Members	Mechanism of action	Remarks
Blue-green algae	Anabaena, Aphanizomenon, Microcystis	Microcystin LR (cyclic heptapeptide)	More closely related to bacteria
Pyrrolizidine alkaloids	Senecio, Cynoglossum, Crotalaria, Heliotropium	Ingested alkaloids converted to pyrrolic esters by cytochrome p450 enzymes	Esters are alkylating agents that act on cytosolic and nuclear proteins. Megalocytes due to antimitotic effect.
Aflatoxin	*Aspergillus flavus* Aflatox in B_1 (toxic intermediates produced by cytochrome p450 enzymes)		Toxin and carcinogen. Sheep more resistant.
Sporidesmin	*Pithomyces chartarum* (fungus growing on dead rye grass)	Necrosis of the epithelium of large intrahepatic and extrahepatic biliary ducts	Results in cholestasis with failure to excrete phylloerythrin leading to photosensitization
Mushroom	*Amanita* sp.	Toxic cyclopeptides Palloidin (toxic heptapeptide)	Inhibition of RNA polymerase II function disrupting DNA and RNA transcription Disruption of intracellular actin filaments

nalicular pumps that normally secrete bile into the canaliculi. This disruption results in cholestasis. (estrogen, erythromycin)
6. Direct damage to mitochondria decreases production of adenosine triphosphate as well as resulting in the release of cytochrome-c leading to apoptosis or necrosis. (antiviral nucleosides, intravenous tetracycline)

Certain toxic compounds may affect cells other than hepatocytes.[2] Damage to biliary epithelium may be caused by trimethoprim-sulfa or sporidesmin, while damage to Kupffer cells can be caused by endotoxin. Arsenicals damage endothelial cells of the liver, and vitamin A excess causes activation of hepatic stellate cells.

Equine serum hepatitis is an idiopathic condition most closely associated with administration of equine-origin biologics.[1,3] It is generally reported 41-60 days following administration of a biologic product, and is characterized by acute hepatic centrilobular necrosis.[1]

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References:

1. Aleman M, Nieto JE, Carr EA, Carlson GP: Serum hepatitis associated with commercial plasma transfusion in horses. J Vet Intern Med 19:120-122, 2005
2. Cullen JM: Liver, biliary system, and exocrine pancreas. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 440-447. Elsevier, St. Louis, MO, 2007
3. Guglick MA, MacAllister CG, Ely R W, Edwards W: Hepatic disease associated with administration of tetanus antitoxin in eight horses. J Am Vet Med Assoc 11:1737-1740, 1995
4. Stalker MJ, Hayes MA: Liver and biliary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 322-333. Elsevier Limited, St. Louis, MO, 2007
5. Toshach K, Jackson MW, Dubielzig RR: Hepatocellular necrosis associated with the subcutaneous injection of

an intranasal *Bordetella bronchiseptica*-canine parainfluenza vaccine. J Am Anim Hosp Assoc 33:126-128, 1997

CASE II – S61/07 (AFIP 3063785).

Signalment: Two and a half-year-old, male beagle, canine.

History: The dog died with multiple bite wounds inflicted by other dogs kept in the same kennel.

Gross Pathology: At necropsy the animal displayed multifocal, severe epidermal ulcers and excoriations of the skin of the neck, thorax and both hind limbs associated with multifocal subcutaneous and intramuscular hemorrhages. The mandibular and retropharyngeal lymph nodes were enlarged and severely hyperemic. The endocardium had multiple petechial hemorrhages and the atrioventricular valves had mild nodular endocardiosis. The liver and lung were moderately congested. In addition, the lung had mild alveolar edema and emphysema.

Histopathologic Description: Within the kidney there was multifocal vacuolation, degeneration and necrosis with sloughing and loss of tubular epithelial cells. Epithelial degeneration and necrosis were frequently associated with small cytoplasmic granular deposition of a brown-greenish pigment. Tubuli were multifocally moderately dilated and contained hyalin or coarsely granular eosinophilic to brown-greenish casts **(Fig 2-1)**. The Bowman´s capsule spaces contained abundante osinophilic, proteinaceous material. Interstitial and glomerular blood vessels were moderately congested with multifocal prominent dilation of cortical veins.

A Turnbull blue stain identified iron in the tubular casts, the brush border and in cytoplasmic granular deposits of the tubular epithelium, consistent with chromoproteinuria.

Contributor's Morphologic Diagnosis: Kidney: Tubular degeneration and necrosis **(Fig. 2-2)**, acute, moderate, multifocal with cytoplasmic pigment deposition and intratubular chromoprotein casts.

Contributor's Comment: The lesions are consistent with acute tubular necrosis following traumatic rhabdomyolysis and chromoproteinuria.
Myoglobinuria as a consequence of elevated myoglobin serum concentration can be seen in metabolic dysfunction (e.g. equine exertional rhabdomyolysis, tying up), stress (e.g. capture myopathy) or severe direct trauma to muscles. In cases of traumatic injury, animals commonly also have renal hypoperfusion due to hypovolemic shock. The proposed mechanisms involved in myoglobinuria-induced renal injury include renal vasoconstriction, intraluminal cast formation and direct intra- and/or extracellular toxicity of myoglobin.

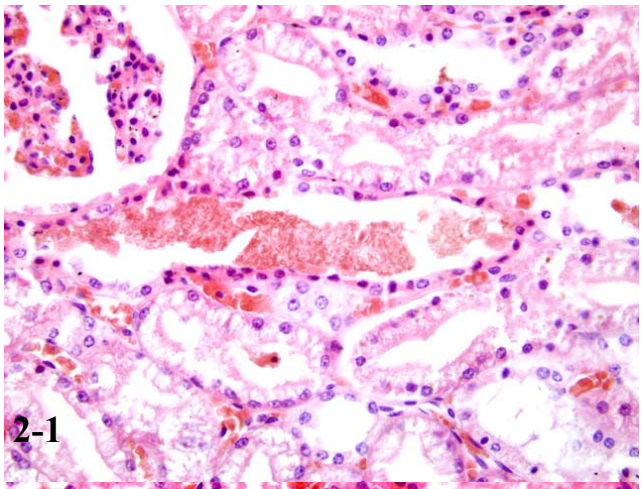
It has been argued that renal vasoconstriction is due to extravasation of fluid in areas of damaged muscle tissue leading to intravascular volume depletion. Furthermore, activation of cytokine cascades and scavenging of nitric oxide as an important endogenous vasodilatator by heme protein contribute to renal hypoperfusion.

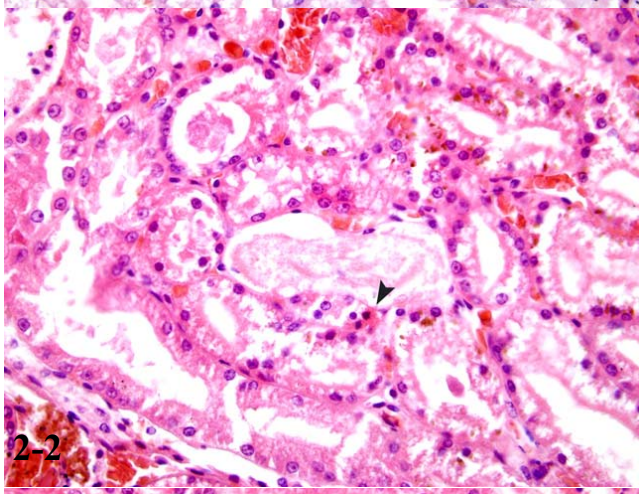
In contrast to earlier views, it has been shown that the intraluminal cast formation during myoglobinuria enhances the dose dependent toxicity of heme by its accumulation and uptake and not by intratubular obstruction. Additionally, hypovolemia, renal vasoconstriction and loss of myoglobin solubility in acidic urine facilitate the formation of casts.

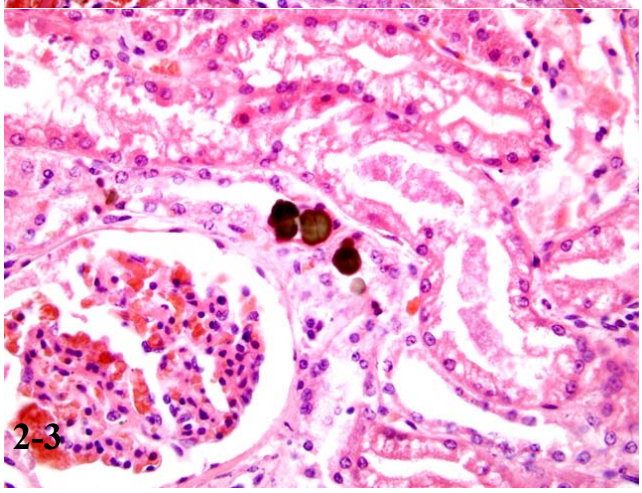
The exact mechanisms of direct myoglobin toxicity are still under investigation. It has been hypothesized that by heme protein endocytosis, tubular plasma membranes become more vulnerable to the effects of phospholipase A_2. Furthermore, iron dependent mechanisms of cellular damage including formation of free radicals, subsequent oxidative stress and lipid peroxidation have been proposed.

Acute tubular necrosis is the most common reason for acute renal failure. Early degenerative lesions commonly seen with acute renal failure include loss of brush borders, flattening of the epithelium, detachment of cells, disruption of tubular basement membranes, formation of intratubular casts, and dilation of the lumens. These changes are observed predominantly in proximal tubules, but injury can also be demonstrated in the distal nephron and may progress to signs of necrosis like hypereosinophilia and loss of cellular detail. The distal nephrons seem to be secondarily damaged by obstruction with desquamated cells, cellular debris, hemoglobin, myoglobin, and other plasma proteins. Tubular regeneration, represented by flattened to elongated epithelial cells with hyperchromatic nuclei and mitosis can be seen after about three days. Within 2-3 weeks after toxin exposure, recovery of normal renal structure may be completed.

Other conditions damaging renal tubular epithelium may result in morphologic changes similar to the lesions de-







scribed here. However, the pigment deposition seen in this case is regarded as specific for hemoglobinuria, myoglobinuria or bilirubinuria. Other common nephrotoxins producing specific acute tubular necrosis in domestic animals include heavy metals (e.g. mercury, lead, arsenic), antibiotics, antifungal agents, anti-inflammatory drugs, and fungal, bacterial and plant toxins.

AFIP Diagnosis: 1. Kidney: Degeneration and necrosis, tubular, acute, multifocal, moderate, with orange-red-brown casts, Beagle (*Canis familiaris*), canine.
2. Kidney: Anisotropic green-brown crystals, intratubular, multifocal **(Fig. 2-3).**

Conference Comment: The contributor gives an excellent overview of myoglobinuric nephrosis. Hemoglobin and myoglobin are chromoproteins that have been associated with hemoglobinuric nephrosis or myoglobinuric nephrosis respectively. Hemoglobin is normally bound to the carrier protein haptoglobin, which is too large to be filtered by the glomerulus. Therefore, hemoglobin is not excreted in the urine unless supplies of the carrier molecule are depleted. Hemoglobin and myoglobin have little nephrotoxicity by themselves [4,6], but when associated with renal ischemia, acidic urine, and decreased glomerular filtration rate, they contribute to acute renal failure.[6]

It is generally accepted that vasoconstriction, lipid peroxidation, and acidification of the urine all play roles in acute tubular necrosis. Cast formation is thought to result from decreased urine flow associated with a decreased GFR.[1,3] In vitro studies of myoglobin toxicity in Fischer 344 rats suggest primary mechanisms of damage result from diminished pyruvate-stimulated gluconeogenesis, decreased total glutathione levels and induction of lipid peroxidation.[5] The exact mechanisms for these actions and their effect in vivo are not fully known.

Hematuria, hemaglobinuria, and myoglobinuria will all generate a positive occult blood test. They can be differentiated by various diagnostic tests.[2] Centrifugation will cause sedementation of erythrocytes leaving a clear supernatant with hematuria. Red-brown urine that does not clear upon centrifugation may be either hemoglobinuria or myoglobinuria. These may be differentiated by adding saturated ammonium sulfate solution, which will precipi-

2-1. Kidney,Beagle. Numerous ectatic tubules and ducts contain moderate amounts of red-orange granular casts. Often these tubules are lined by attenuated epithelium. (HE 400X).
2-2. Kidney, Beagle. There is multifocal tubular epithelial necrosis characterized by hypereosinophilic, shrunken epithelial cells with pyknotic nuclei (arrowhead). Multifocally within the interstitium there is mild hemorrhage. (HE 400X).
2-3. Kidney, Beagle. Few tubules contain variably- sized, green-brown, irregularly round crystals. (HE 400X).

Pigmentary changes in the kidney, extracted from Maxie et al.[4] and Newman et al.[6]

Condition	Pigment	Gross lesion	Histologic lesion
Hemoglobinuric nephrosis (acute hemolytic crisis)	Hemoglobin	Dark red-brown to blue-black with radial streaks	Fine red granular speckling within epithelial cells or granular casts
Myoglobinuric nephrosis (acute rhabdomyolysis)	Myoglobin	Dark red-brown to blue-black with radial streaks	Fine red granular speckling within epithelial cells or granular casts
Hemosiderosis (chronic hemolytic anemia)	Hemosiderin	Brown discoloration of cortex	Pigment within the epithelial cells of proximal tubules
Cloisonné kidney (non-clinical condition)	Ferritin and hemosiderin	Brown to black renal cortices	Brown pigmentation of basement membrane, convoluted portions of proximal tubules
Lipofuscinosis Brown	iron-free pigments	Radial dark lines on the cut surface of cortex, sparing the medulla	Fine brown granules in epithelial cells of convoluted tubules

tate hemoglobin. A clear supernatant following ammonium sulfate addition is indicative of hemoglobinuria, while a red-brown color indicates myoglobinuria.

The green-brown intratubular crystals were identified by scanning electron microscopy with energy dispersive x-ray analysis (SEM-EDXA) and infrared spectroscopy (IR) as consistent with calcium oxalate monohydrate. The calcium oxalate crystals in this case are unusual in appearance because of the green-brown color in H&E. The crystals stained positive for Von Kossa and negative for Alizarin red. It is possible that protein and iron deposition within the crystals could account for their abnormal appearance. We would like to thank the AFIP Department of Environmental and Toxicologic Pathology for their assistance in evaluating this case.

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References:
1. Efstratiadis G, Voulgaridou A, Nikiforou D, Kyventidis A, Kourkouni E, Vergoulas G: Rhabdomyolysis updated. Hippokratia 11:129-137, 2007
2. Gregory CR: Urinary system. In: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 240-241. Iowa State Press, Ames, Iowa, 2003
3. Holt S, Moore K: Pathogenesis of renal failure in rhabdomyolysis: the role of myoglobin. Exp Nephrol 8:72-76, 2000
4. Maxie MG, Newman SJ: The urinary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 475476. Elsevier Limited, St. Louis, MO, 2007
5. Minigh JL, Valentovic MA: Characterization of myoglobin toxicity in renal cortical slices form Fischer 344 rats. Toxicol 187:77-87, 2003
6. Newman SJ, Confer AW, Panciera RJ: Urinary system. In: Pathologic Basis of Disease, McGavin MD and Zachery JF, 4th ed., pp. 644-658. Mosby Elsevier, St. Louis, Missouri, 2007
7. Zager RA: Rhabdomyolysis and myohemoglobinuric acute renal failure. Kidney Int 49:314-326, 1996

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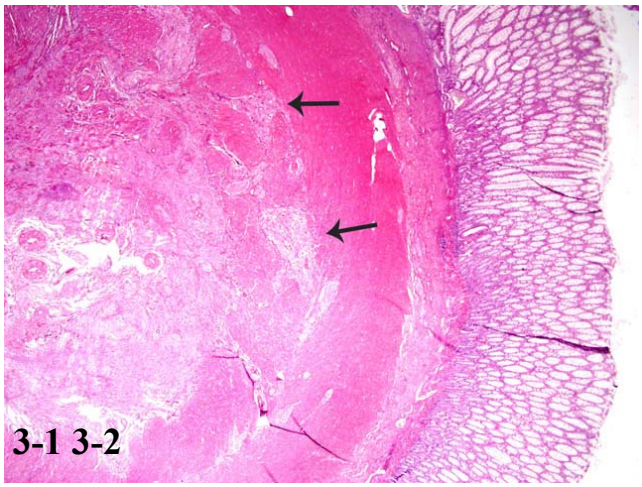
CASE III – CAS 2 (AFIP 2991412).

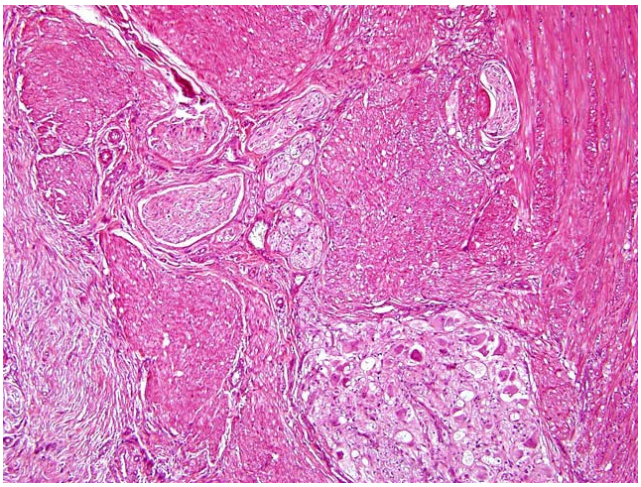
Signalment: 1-year-old, male, Beagle dog.

History: This dog was part of a 10-day oral toxicological study and was euthanized at the end of the study. There were no relevant clinical signs.

Gross Pathology: An abnormal shape of the cecum was the only relevant macroscopic finding.

Histopathologic Description: There is invagination of





3-1. Cecum, Beagle. Tthere are increased numbers of large, irregularly shaped ganglia (arrows) within the tunica muscularis and serosa of the intussusceptum. (HE 100X).
3-2. Cecum, Beagle. Higher magnification demonstrating increased numbers of nerve bundles and ganglia. (HE 200X).

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the tip of the cecum within its lumen. All parts of the cecum wall are diffusely, moderately thickened (about twice normal thickness). The muscularis mucosa, the submucosa, some part s of t he m uscular l ayers (particularly the longitudinal layer), and the serosa are replaced by a poorly demarcated tissue, primarily in the same location as the myenteric (Auerbach's) and the submucous (Meissner's) plexuses **(Fig. 3-1)**. This tissue is composed of irregularly-arranged wavy fascicles of nerve fibers with round and spindle cells, and some clusters of enlarged ganglion cells **(Fig. 3-2)**. The mucosa is moderately hyperplastic, with multifocal to coalescing hemorrhages in the lamina propria, and multifocal minimal degeneration of some glands. Scattered in the submucosa and the proliferative neural tissue are some cells containing large pigmented brown granules (hemosiderin).

Contributor's Morphologic Diagnoses: Cecum: Transmural ganglioneuromatosis, locally extensive, with intussusception.

Contributor's Comment: Intestinal ganglioneuromatosis refers to a hyperplastic proliferation of ganglion cells, nerve fibers, and supporting cells of the enteric nervous system. In humans, intestinal ganglioneuromatosis is most often part of multiple tumor syndromes, particularly the multiple endocrine neoplasia (MEN) 2B syndrome.[12] MEN-2B is inherited in an autosomal dominant fashion and is caused by a single mutation in the RET proto-oncogene. This heritable endocrine disorder is characterized by medullary thyroid carcinoma, pheochromocytoma, multiple mucosal neuromas, gastrointestinal ganglioneuromatosis, corneal nerve thickening and skeletal abnormalities.[8] Gastrointestinal symptoms are common in patients with MEN-2 B, and are secondary to the pseudo-obstruction caused by the ganglioneuromatosis.[6] The pathogenesis of ganglioneuromatosis is not well understood, but some studies in humans indicate that it may be related to the overproduction of some nerve growth factors.

Immunohistochemically, some cases of ganglioneuromatosis were shown to be a complex hyperplasia of several peptidergic, cholinergic, and probably adrenergic nerve fibers instead of a selective overgrowth of one type of nerve fibers.[4]

Some rare cases of intestinal ganglioneuromatosis or ganglioneuromas have been reported, most often in young animals: in a horse[1], a steer[3], a cat[9], and 3 dogs.[5,11,13] In all cases, there were clinical signs (e.g. colic, impaction, anorexia, vomiting, diarrhea, rectal prolapse) that led to surgical resection of the masses. Masses were located in the small intestine (3 cases), colon (1 case), colorectum (2 cases) or Vater's papilla (1 case). This is the first reported case of asymptomatic ganglioneuromatosis in a dog.

AFIP Diagnosis: Cecum (per contributor): Ganglioneuromatosis, with tussusception, Beagle (*Canis familiaris*), canine.

Conference Comment: Ganglioneuromas are composed of mature autonomic ganglion cells, satellite cells, un-

myelinated and occasionally myelinated axons, Schwann cells and a fibrous stroma. They are generally considered benign neoplasms. Intestinal ganglioneuromatosis is considered a hyperplasia of similar elements; it is typically transmural. As noted by the contributor, both lesions are rare in animals and have not been found to be associated with MEN-like syndromes.[7] Some have suggested that ganglioneuromas may actually represent hamartomas (benign, nonneoplastic, tumor-like nodules consisting of an overgrowth of mature cells that normally occur in the affected organ) rather than beni gn neoplasms.[10]

Intussusceptions are described as having three layers: (1) outer wall of the receiving segment, (2) middle returning segment of invaginated bowel, and (3) inner entering segment. The intussusception seen in this lesion is unusual in that it contains only two of the three layers, a feature that will occur only through the invagination of a blind pouch (in this case, the tip of the cecum). Cecal inversion is another term for such a lesion **(Fig. 3 -3)**. Various causes of intussusception may include linear foreign bodies, heavy parasitism, previous intestinal surgery, enteritis, and intramural lesions. It may also develop as a terminal, agonal or postmortem event.[2]

We appreciate the assistance from the Departments of Gastrointestinal Pathology, Neuropathology, and Soft Tissue Pathology at the Armed Forces Institute of Pathology in consultation on this case.

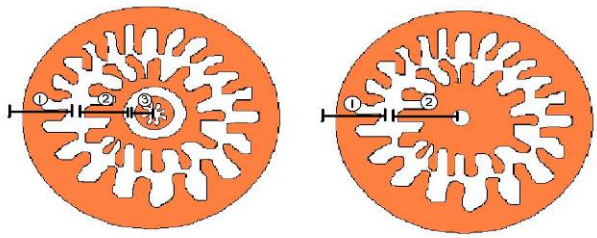

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Fig 3-3. A. Intussusception of tubular section of bowel consisting of three layers: (1) outer wall of the receiving segment, (2) middle returning, segment of invaginated bowel, and (3) inner entering segment.
B. Intussusception of a blind pouch consisting of two layers: (1) outer wall of the receiving segment, and (2) the middle returning, segment of invaginated bowel.

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References:

1. Allen D, Swayne D, Belknap JK: Ganglioneuroma as a cause of small intestinal obstruction in the horse: a case report. Cornell Vet 79:133-141, 1989
2. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 96-97. Elsevier Limited, St. Louis, MO, 2007
3. Cole DE, Migaki G, Leipold HW: Colonic ganglioneuromatosis in a steer. Vet Pathol 27:461-462, 1990
4. D'Amore ES, Manivel JC, Pettinato G, Niehans GA, Snover DC: Intestinal ganglioneuromatosis: mucosal and transmural types. A clinicopahologic and immunohistochemical study of six cases. Hum Pathol 22:276-286, 1991
5. Fairley RA, McEntee MF: Colorectal ganglioneuromatosis in a young female dog (Lhasa Apso). Vet Pathol 27:206-207, 1990
6. Goyal RK, Hirano I: The enteric nervous system. N Engl J Med 334:1106-1115, 1996
7. Head KW, Else R W, Dubielzig: Tumors of the alimentary tract. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., p. 470. Blackwell Publishing, Ames, IA, 2002
8. Komminoth P: Multiple endocrine neoplasia type 1 and 2: from morphology to molecular pathology. Vehr Dtsch Ges Path 81:125-138, 1997
9. Patnaik AK, Lieberman PH, Johnson GF: Intestinal ganglioneuroma in a kitten – a case report and review of literature. J Small Anim Pract 19:735-742, 1978
10. Porter BF, Storts RW, Payne HR, Edwards JF: Colonic ganglioneuromatosis in a horse. Vet Pathol 44:207-210, 2007
11. Ribas JL, Kwapien RP, Pope ET: Immunohistochemistry and ultrastructure of intestinal ganglioneuroma in a dog. Vet Pathol 27:376-379, 1990
12. Shekitka KM, Sobin LH: Ganglioneuromas of the gastrointestinal tract. Relation to Von Recklinghausen disease and other multiple tumor syndromes. Am J Surg Pathol 18:250-257, 1994
13. Van Den Ingh TSGAM, Rothuizen J: Ganglioneuroma of Vater's papilla and extrahepatic cholestasis in a dog. Vet Pathol 21:254-256, 1984

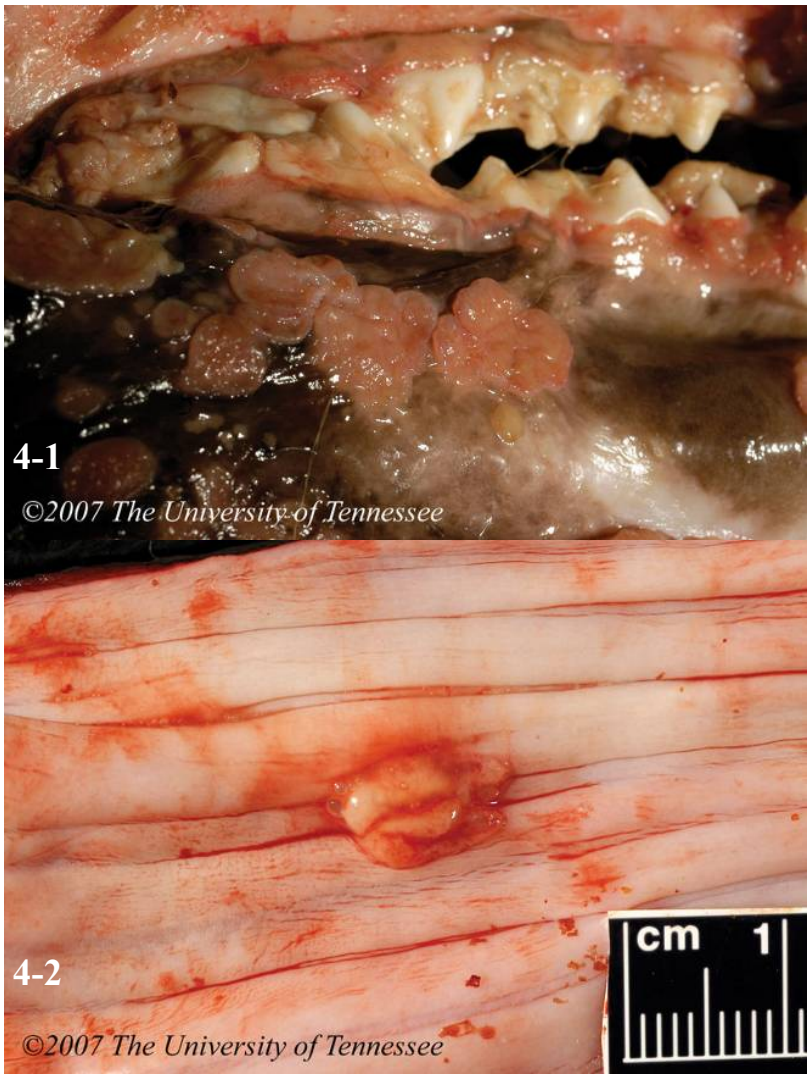
CASE IV - 07-45 (AFIP 3074806).

Signalment: Seven-month-old, female, Golden Retriever mixed breed, *Canis familiaris*, dog

History: The dog was presented with chronic conjunctivitis, gingival lesions, and respiratory disease. The clinical signs had begun at 4.5 weeks of age and had progressed. Previous diagnostics including conjunctival biopsies, cytology and bacterial culture of conjunctival swabs, canine distemper serology, virus isolation, and routine bloodwork failed to establish a diagnosis. On presentation there were circular, raised, pink, fleshy, mucosal lesions of the conjunctiva, throughout the oral cavity and naso-pharynx and an ulcer on the soft palate. Thoracic auscultation revealed harsh referred upper airway sounds. A repeat biopsy of the ocular conjunctiva identified a profuse accumulation of fibrin in areas of ulceration and under-running the epithelium. A diagnosis of ligneous conjunctivitis was made. Based on this diagnosis and involvement of other mucosal surfaces, a presumptive diagnosis of plasminogen deficiency was made. This was confirmed by a low plasminogen functional activity assay of 35% (compared to a normal age-matched control of 111 % and pooled samples from normal dogs of 118%). The conjunctival lesions recurred after the excisional biopsy. A 2-week round of topical and intravenous treatment with fresh frozen plasma diminished the conjunctival lesions; however, four weeks later the dog had a lower plasminogen activity assay (10%), weight loss, inappetance and lethargy. The owners requested euthanasia.

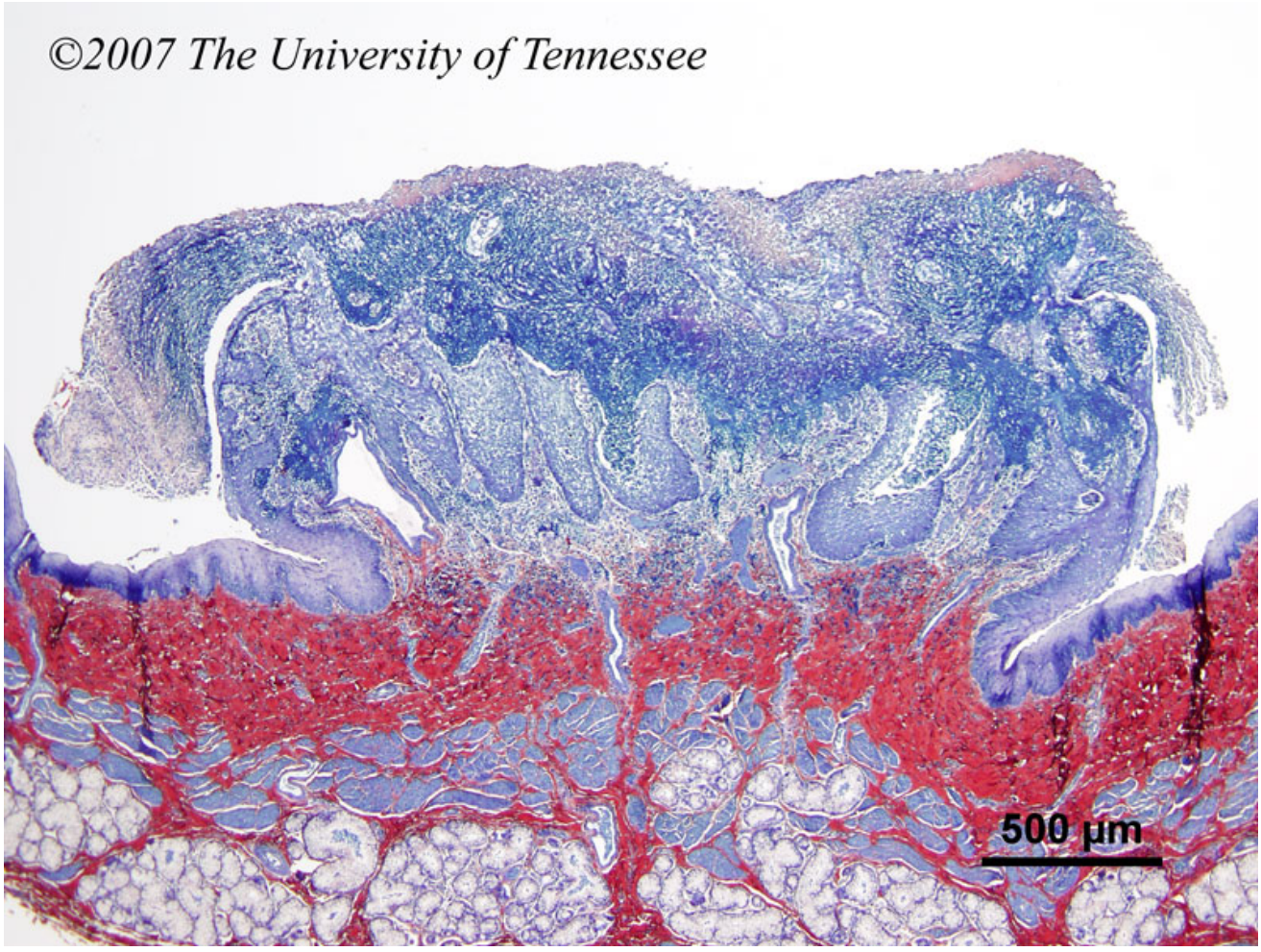
Gross Pathology: Multifocal to coalescing, 0.2cm to 2.0 cm in diameter, raised, white to gray, granular, plaques decorated the glossal, buccal and gingival surfaces **(Fig 4-1)**. In the soft palate, there was a 2 x 2cm ulcerated area, covered by a thick layer of a granular yellow material. There were multifocal, 0.5 cm to 1 cm diameter, gray plaques on the esophageal mucosa **(Fig. 4-2)**. Gray to yellow, granular, fibrinous plaques were disseminated over the length of the tracheal mucosa. Multifocally slightly elevated plaques covered the epicardium of the right and left ventricles. Additionally there was mild hydrocephalus, rare intestinal mucosal hemorrhages, and a mild fibrinous perihepatitis

Histopathologic Description: The sections of esophagus submitted have focal erosion to ulceration of locally hyperplastic epithelium covered by an exophytic coagulum of fibrin and cellular debris **(Fig 4-3)**. The exophytic coagulum is supported by a pedunculated to broad base of fibrin irregularly infiltrated by granulation tissue



4-1. Oral cavity, Golden retriever mix, canine. Raised, white to gray, granular, plaques decorate the glossal, buccal and gingival surfaces.
4-2. Esophagus, Golden retriever mix, canine. 0.5cm to 1cm diameter gray plaques on the esophageal mucosa. →

Gross photographs courtesy of The University of Tennessee, Collage of Veterinary Medicine, 2407 River Dr., Knoxville, TN, 37996



4-3. Esophagus, Golden retriever mix, canine. Exophytic plaque composed of markedly thickened lamina propria which elevates the overlying moderately hyperplastic and ulcerated epithelium. Superficially, these plaques are covered by a fibrinocellular mat. (PTAH).

Photomicrograph courtesy of The University of Tennessee, Collage of Veterinary Medicine, 2407 River Dr., Knoxville, TN, 37996

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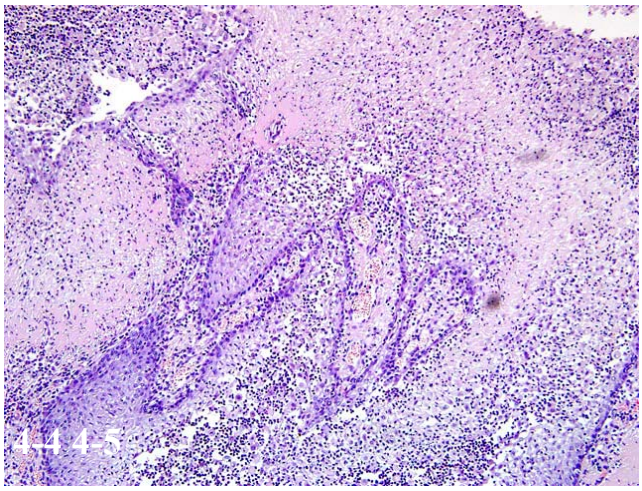
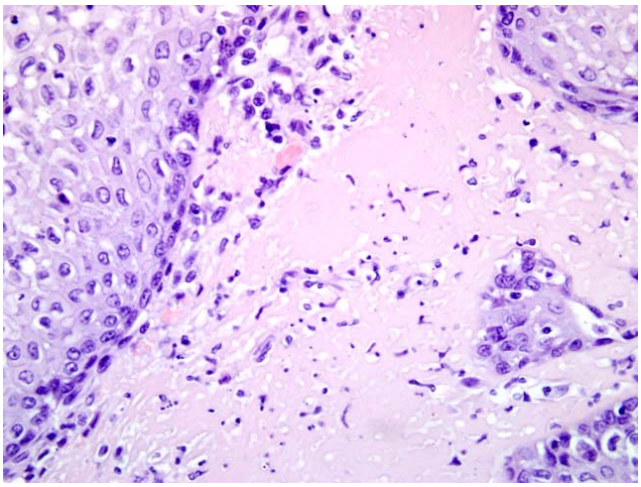
with macrophages and neutrophils and a few lymphocytes, plasma cells and eosinophils admixed. In some areas, this thick layer of fibrin and inflammatory cells forms finger-like projections partially overlain by squamous epithelium **(Fig. 4-4)**. In immediately adjacent esophagus there is limited focal to multifocal subepithelial fibrin deposition **(Fig. 4-5)**.

Contributor's Morphologic Diagnosis: Severe, chronic, ulcerative and proliferative, fibrinomembranous esophagitis

Contributor's Comment: This patient was initially presented to the Ophthamology service because of bilateral conjunctival lesions. The clinical diagnosis of ligneous conjunctivitis was made based on the histologic appearance of the conjunctival biopsy and functional plasminogen activity assay. This form of conjunctivitis is so named because of the wood-like consistency of the membranes. Reports of this condition in canines are rare, predominantly in the Doberman Pinscher breed.[9] It is more commonly reported in females in both the veterinary and human literature.[5,9] The condition is linked to a type I plasminogen deficiency and an autosomal-recessive genetic mutation has been identified as a common cause of this functional deficiency.[10] The pathogenesis of the lesions in the conjunctiva and other mucosal sites involves the coagulation of fibrin following minor mechanical





4-4. Esophagus, Golden retriever mix, canine. Diffusely, overlying the ulcerated plaques, is a thick fibrinous mat admixed with numerous inflammatory cells. (HE 200X).
4-5. Esophagus, Golden retriever mix, canine. Subepithelial fibrin deposition admixed with neutrophils, lymphocytes, and macrophages. (HE 400X).

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injury to tissues. This fibrin rich matrix provides hemostasis and is subsequently replaced by granulation tissue in normal individuals.

Impaired proteolysis due to deficiency in plasminogen results in an inability to remove the fibrin rich matrix and remodel granulation tissue, thus arresting wound healing at the granulation tissue stage and resulting in the accumulation of fibrin rich membranes.

The condition is typically diagnosed in neonates but may develop at any age. The palpebral conjunctiva is affected most frequently but the bulbar conjunctiva and cornea may be affected as well. Other mucosal sites such as the gingiva, ear, respiratory tract, gastrointestinal tract and female reproductive tract may also be affected with or without the presence of conjunctival lesions.[3,4,9,12] As was the case in this dog, hydrocephalus has been reported in infants with plasminogen deficiency.[4,12] In this case there were multiple venous thrombi associated with lesions in the oral cavity and present in the pulmonary field. While the primary respiratory signs in this dog were likely due to tracheal obstruction, the pulmonary thrombi may have played some role in this animal's respiratory condition. Although not reported in the human literature in association with ligneous conjunctivitis, severe decreases in plasminogen activity, when coupled with other insults or precipitating events such as operations, trauma or infection have been reported to increase the risk for thromboembolic events.[8]

AFIP Diagnosis: Esophagus: Esophagitis, proliferative, fibrinous, neutrophilic and lymphoplasmacytic, multifocal, marked, with ulceration, acantholysis, granulation tissue and multifocal subepithelial fibrin, Golden retriever mix (*Canis familiaris*), canine.

Conference Comment: The contributor gives an excellent overview of plasminogen deficiency associated with ligneous conjunctivitis. Conference participants are encouraged to review the article on this case published by Johnstone McLean et al.[7] Plasminogen plays a vital role in intravascular and extravascular fibrinolysis, wound healing, cell migration, tissue remodeling, angiogenesis, and embryogenesis.[2] Plasminogen may be converted to plasmin by cleavage with either tissue-type plasminogen activator (tPA) leading to lysis of fibrin clots in the blood stream or urokinase-type plasminogen activator (uPA) associated with wound healing and tissue remodeling.[1]

It is interesting to note that in humans and animals diagnosed with type I plasminogen defficiency, there is little to no increase in the risk of developing intravascular thrombosis, which implies the existence of an alternative pathway for intravascular fibrinolysis.[7,11,13]

The pseudomembranous deposits on mucous membranes occurs primarily in areas of previous damage. The hyaline material may contain scattered neutrophils, eosinophils, T-lymphocytes, plasma cells, mast cells and/or foreign material. Immunohistochemistry may be positive for fibrin, albumin and immunoglobulins (IgG, IgA).[3,6]

Contributor: The University of Tennessee, College of Veterinary Medicine, 2407 River Dr., Knoxville, TN, 37996

References:

1. Bugge TH, Flick MJ, Danton MJ, Daugherty CC, Romer J, Danao K, Carmeliet P, Collen D, Degen JL: Urokinase-type plasminogen activator is effective in fibrin clearance in the absence of its receptor or tissue-type plasminogen activator. Proc Natl Acad Sci USA 93:5899-5904, 1996
2. Castellino FJ, Ploplis VA: Structure and function of the plasminogen/plasmin system. Thromb Haemost 93:647-654, 2005
3. Chai F, Coates H: Otolaryngeal manifestations of ligneous conjunctivitis. Int J Pediatr Otorhinolaryngol 22:760-762, 2003
4. Cifti E, Ince E, Akar N, Dogru U, Tefs K, Schuster V: Ligneous conjunctivitis, hydrocephalus, hydrocele and pulmonary involvement in a child with homozygous type I plasminogen deficiency. Eur J Pediatr 162:462-465, 2003
5. Cohen SR: Ligneous conjunctivitis: an opthalmic disease with potentially fatal tracheobronchial obstruction. Am Otol Rhinol Laryngol 99:509-512, 1990
6. Wilcock BP: Eye. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, p. 481. Elsevier Limited, St. Louis, MO, 2007
7. Johnstone McLean NS, Ward DA, Hendrix DV, Donnell RL, Ilha MR: Ligneous conjunctivitis secondary to a congenital plasminogen deficiency in a dog. J Am Vet Med Assoc 232:715-721, 2008
8. Lottenberg R, Dolly JR, Kitchens CS: Recurrent thromboembolic disease and pulmonary hypertension associated with severe hypoplasminogenia. Am J Hematol 168:181-193, 1985
9. Ramsey DT, Ketring KL, Glaze MB, Knight B, Render JA: Ligneous conjunctivitis in four doberman pinschers. J Am Anim Hosp Assoc 32:439-447, 1996
10. Schuster V, Zeitler P, Seregard S, Ozcelik U, Anadol D, Lutchman-Jones L, Meire F, Mingers AM, chambeck C, Kreth HW: Homozygous and compound–heterozygous type I plasminogen deficiency is a common cause of ligneous conjunctivitis. Thromb Haemost 85:1004-1010, 2001
11. Schuster V, Hugle B, Tefs K: Plasminogen deficiency. J Thromb Haemost 5:2315-2322, 2007
12. Scully C, Allen C, Ervesen G, Hodgson T, Speight P: Oral lesions indicative of plasminogen deficiency (hypoplasminogenemia). Oral Surg Oral Med Oral Pathol Radiol Endod 91:334-337, 2001
13. Shigekiyo T, Uno Y, Tomonari A, Satoh K, Hondo H, Udea S, Saito S: Type I congenital plasminogen deficiency is not a risk factor for thrombosis. Thromb Haemost 67:189-192, 1992