



WEDNESDAY SLIDE CONFERENCE 2015-2016

Conference 8

11 November 2015

CASE I: 14-292 (JPC 4066249).

Signalment: 20-year-old female pony
(*Equus caballus*)

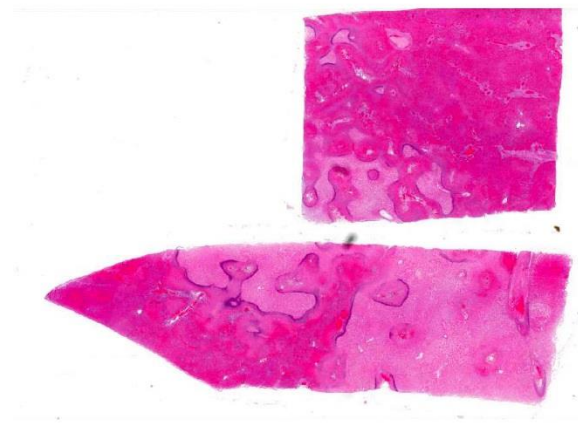


Liver, pony: Multifocally, the hepatic parenchyma is dry, malodorous, and has small pockets of emphysema. (Photo courtesy of: Photo courtesy of: University of Calgary Faculty of Veterinary Medicine, Clinical Skills Building, 11877 85th St NW, Calgary, AB T3R 1J3. <http://www.vet.ucalgary.ca/>)

History: A 20-year-old female pony presented to the referring veterinarian with a history of acute onset depression, decreased appetite, and separation from herdmates. On initial presentation, the pony was tachycardic, tachypneic, and mildly febrile. Over a 2-day period the pony deteriorated with development of a high fever and marked icterus. Complete blood count revealed lymphopenia, mild monocytosis and mild thrombocytopenia. Marked increases in SDH, AST, ALP and GGT were noted on

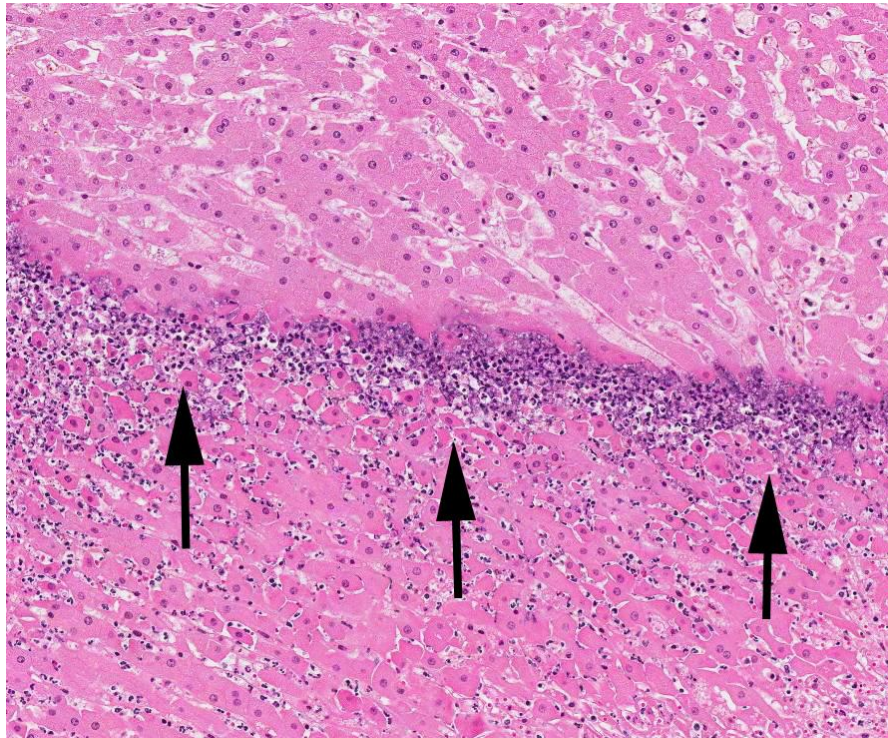
chemistry. Abdominal ultrasound showed multifocal, hyperechoic areas within the liver and a 4 cm mass in close proximity to the spleen. Treatments included intravenous fluids, antibiotics, and anti-inflammatories. The pony was humanely euthanized 3 days after initial presentation.

Gross Pathology: At necropsy, the body was fresh, in good nutritional condition and there was no evidence of dehydration. There was severe icterus. Within the peritoneal cavity there were liters of opaque, red fluid. On the serosal surface of the small intestines there were numerous petechiae and ecchymoses. Primarily located within the left side of the liver, there was an extensive, mo-



Liver, pony: Two sections of liver exhibit confluent areas of coagulative necrosis. (HE, 5X)

derately well-defined, approximately 35 cm in diameter focus where the parenchyma was swollen, firm and dark red to tan. This area was accompanied by a thick layer of fibrin on the capsular surface with adhesion to the diaphragm and spleen. On cut section, there were multifocal to coalescing areas of pallor and reddening interpreted to be necrosis and hemorrhage, respectively. The hepatic parenchyma was dry, lusterless, and malodorous with multiple small pockets of air (emphysema). Large thrombi were observed



Liver, pony. Areas of coagulative necrosis (top) are delineated by a border of degenerate neutrophils and abundant cellular debris. (HE, 80X)

within blood vessels.

Laboratory Results: Fluorescent antibody testing was negative for *Clostridium chauvoei*, *C. septicum*, *C. novyi* and *C. sordellii*. Significant organisms were not isolated on aerobic or anaerobic cultures. Immunohistochemistry was positive for *C. novyi*.

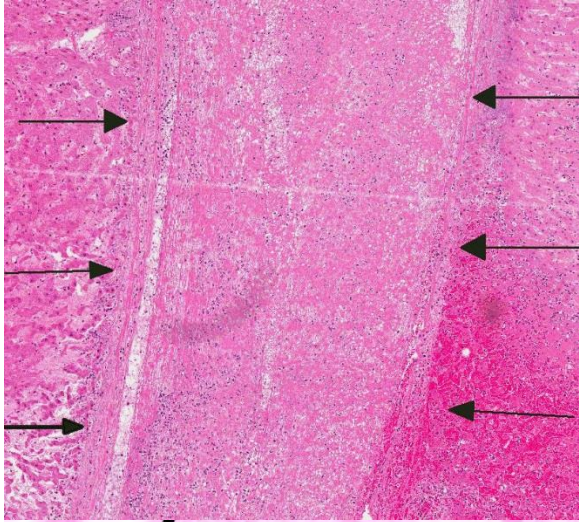
Histopathologic Description: Liver: Involving up to 80% of the hepatic parenchyma in one section, there are multifocal to confluent zones of acute coagulation necrosis which are characterized by hypereosinophilia, loss of nuclear detail, and retention of the tissue architecture. Zones of coagulation necrosis are bordered by an intense band of basophilia which is composed of degenerate neutrophils and nuclear material. Multifocal areas of lytic necrosis are observed and are characterized

by eosinophilic cellular and karyorrhectic debris admixed with degenerate neutrophils, fibrin and hemorrhage. Frequently, the tunica media of blood vessels is disrupted by fibrin, nuclear debris, free red blood cells and neutrophils consistent with vasculitis. Affected blood vessels often contain thrombi. Within the zone of coagulation necrosis there are variable numbers of large (6 μm x 1 μm) bacilli reminiscent of *Clostridium* species. These organisms are gram-positive.

Contributor's Morphologic Diagnosis:

Liver: Hepatitis, necrotizing, extensive, severe, acute with emphysema, necrotizing vasculitis, thrombosis and gram positive bacilli

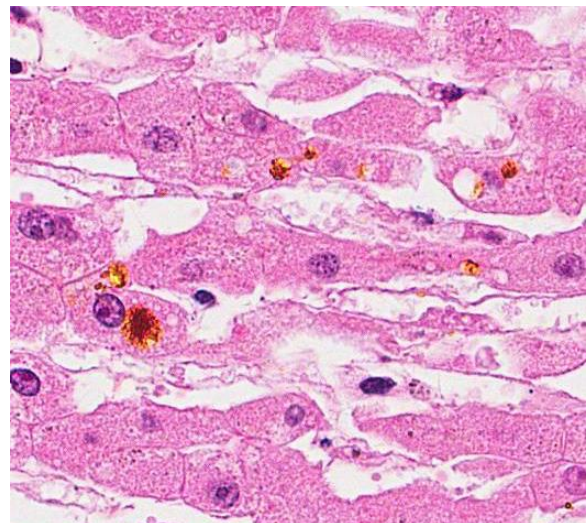
Contributor's Comment: The gross pathology and microscopic lesions in this pony were highly suggestive of clostridial hepatitis, a lesion commonly seen in ruminants, infrequently seen in swine, and rarely reported in equids. In ruminants,



Liver, pony. Blood vessels both within necrotic and viable areas of the parenchyma often contain fibrin thrombi (arrows). (HE, 80X)

Clostridium novyi group of bacteria: black disease (infectious necrotic hepatitis) and bacillary hemoglobinuria.^{5,6} There is considerable overlap in the gross pathology, histopathology and pathogenesis of these diseases. Black disease is caused by *C. novyi* type B, a bacterium that produces potent alpha and beta toxins. Bacillary hemoglobinuria is caused by *C. haemolyticum* (formerly *C. novyi* type D) which produces beta toxin only. Both diseases are characterized by acute hepatic necrosis and other systemic lesions associated with toxemia and generalized vascular damage.⁵ As the name suggests, bacillary hemoglobinuria is further characterized by intravascular hemolysis with anemia and hemoglobinuria.⁶ The pathogenesis of both diseases begins with the ingestion of environmental spores with seeding to histiocytes within the liver, spleen, and bone marrow. Spores lie dormant in the liver until the formation of a localized anaerobic environment allowing for germination of spores and the production of potent exotoxins by vegetative bacteria. In ruminants, migration of the common liver fluke, *Fasciola hepatica*, is thought to be the initiating event.⁶

To date, there are 7 reports of clostridial hepatitis in equids occurring in Australia, New Zealand, the United Kingdom, and the United States.^{3,4,6,7,9} Similar to the current case, clinical disease in horses is characterized by acute onset of depression, fever, abdominal pain, icterus, tachycardia, and tachypnea with rapid deterioration and death in 12-48 hours.^{7,9} Successful therapy has not been described and is not surprising given the rapid course of disease and difficulty in establishing an antemortem diagnosis. Reported necropsy findings include serosanguinous pericardial, pleural and peritoneal effusions, serosal hemorrhages, icterus, fibrinous peritonitis and hepatic necrosis.^{3,4,7,9} The inciting cause of the suitable anaerobic conditions for spore germination within the equine liver has not been definitively determined. Strongyle migration though the liver is an inconsistent finding and in the current case, there was no clear evidence of larval migration.^{5,9} Interestingly, many of the reported cases have a recent history of anthelmintic therapy prior to the onset of clinical signs.^{3,5,9} Recent use of anthelmintics was not reported in the current case.



Liver, pony. Within areas of necrosis, there are small to large aggregates of hematoidin. (HE, 200X)

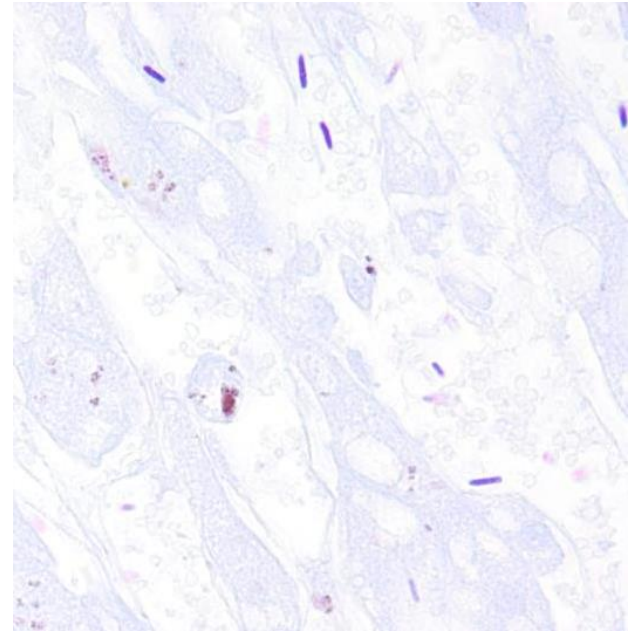
Arriving at an etiologic diagnosis was problematic in the current case. Liver was submitted to two laboratories for anaerobic culture and fluorescent antibody testing (FAT) for *Clostridium* spp. *Clostridium novyi* group organisms were not detected by FAT or by culture, highlighting the challenge at arriving at an etiologic diagnosis in cases of clostridial hepatitis. Both *C. novyi* and *C. haemolyticum* are extremely oxygen sensitive and fastidious in their nutritional requirements making culture challenging and an unreliable diagnostic tool.^{5,9} This diagnostic challenge is further highlighted by a report wherein *C. novyi* type A isolates were sent to 669 laboratories worldwide as part of an external quality control program. Only 3.5% of laboratories made a definitive identification of *C. novyi* type A.² In the current case, immunohistochemistry was positive for *C. novyi* and was instrumental in confirming the diagnosis.

JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal to coalescing, marked with vascular thrombosis.

Conference Comment: The conference histologic description was aligned very closely with the contributor's description, although few participants reported seeing bacilli. A mild amount of emphysema was seen within some sections, but this was not a prominent feature. Interestingly, the areas of coagulative necrosis often did not demonstrate the classic loss of differential staining in hepatocyte nuclei; however, the cells were swollen, pale and largely dissociated from normal hepatic cord architecture. Conference participants generally interpreted the lesion as acute or subacute, which corresponds with the clinical history, and the pattern appeared random rather than having a specific zonal distribution. Reaching an etiologic diagnosis in this case was particularly challenging for participants, with a

variety of possible etiologies discussed, ranging from infectious to toxic.

The differential diagnosis includes equine serum hepatitis, which is a common cause of acute liver failure in young horses. Grossly



Liver, pony. Clostridial bacilli are more easily visualized with a tissue Gram stain. (Gram, 400X) (Photo courtesy of: University of Calgary Faculty of Veterinary Medicine, Clinical Skills Building, 11877 85th St NW, Calgary, AB T3R 1J3. <http://www.vet.ucalgary.ca/>)

in this condition the liver is described as flaccid (“dishrag liver”) with a mottled or reticular pattern on cut section. Histology is characterized by extensive hepatic necrosis with stromal collapse; remaining hepatocytes often demonstrate fatty degeneration. This is typically not an acute process and mild fibroplasia is often present, which is in contrast to the liver seen in this case, although the clinical course has some similarities.¹ Not knowing the age of this animal, another infectious rule out is *C. piliforme*, the cause of Tyzzer’s disease, which is reported in young foals. In contrast to the lesion in this case, the microscopic lesion of Tyzzer’s consists of random foci of coagulative necrosis with neutrophilic infiltrates. The diagnosis depends on seeing the organisms at

the periphery of necrotic areas within degenerate and normal appearing hepatocytes, and the organisms often appear in bundles.¹ *Salmonella typhimurium* was also mentioned as a possibility, but again is most often seen in foals. Lesions are most commonly associated with the intestine, particularly in adult horses, but in cases of septicemia lesions may be seen in the liver.⁸

Since infectious organisms were not apparent in most slides, many conference participants considered a toxic etiology such as blue-green algae. Microcystin-LR is the most commonly referenced cyanobacterial hepatotoxin, found on ponds or other small bodies of water after a seasonal bloom. It is taken up into hepatocytes where it inhibits cytoplasmic protein phosphatases leading to necrosis, apoptosis, and perisinusoidal hemorrhage. The pattern of necrosis is most often centrilobular to massive, but can vary depending on the individual animal and amount of toxin ingested.¹ Overall, this was a challenging case with clinical, gross and histologic characteristics consistent with several potential etiologic agents.

Contributing Institution:

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CASE II: TAMU-02 2012 (JPC 4019891).

Signalment: Yearling, black and white Holstein steer (*Bos taurus*)



A cross section of the brainstem was submitted for microscopic examination. (HE, 5X)

History: Neurologic signs for 6 days, circling, wandering, vocalizing prior to collapsing. Unable to stand, bilateral strabismus, decreased tongue tone.

Gross Pathology: No lesions

Laboratory Results: NA

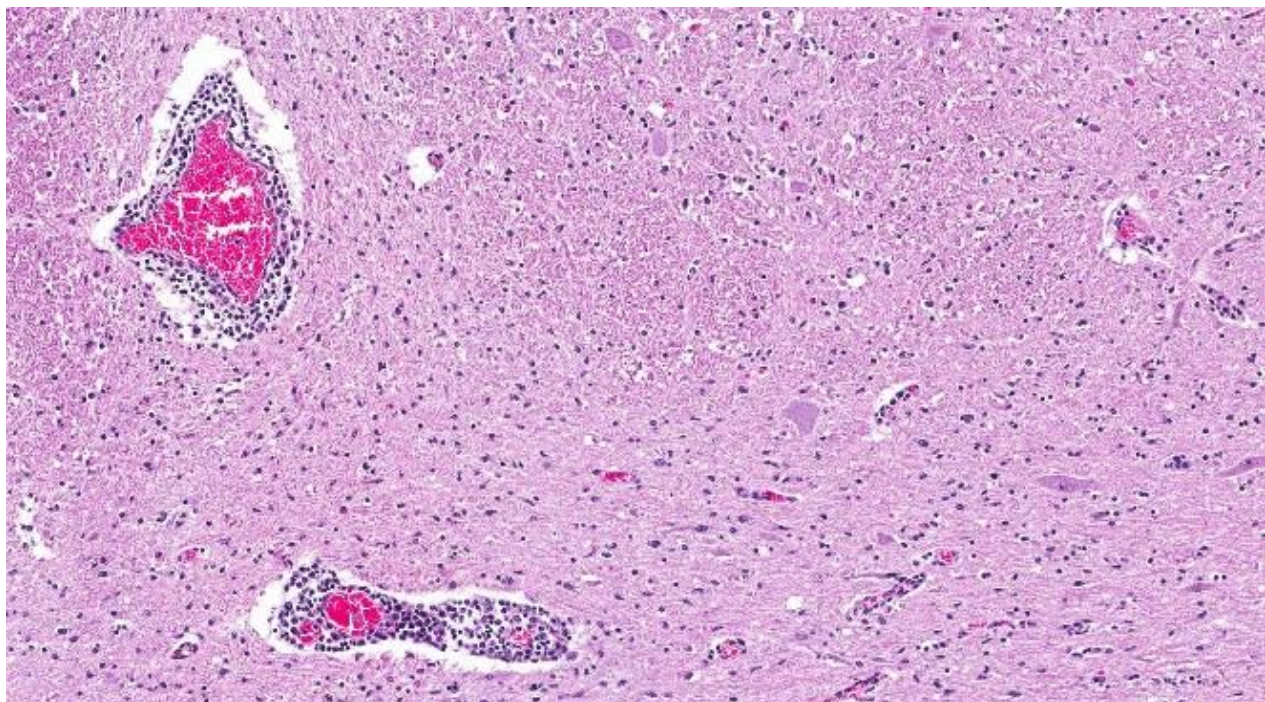
Histopathologic Description: A cross section of medulla oblongata is presented. Perivascular, especially perivenular, cuffs are widespread in the medulla and leptomeninges and vary from mild and single-cell thick to several layers thick. Gliosis is prominent in nuclei and perivascular where the glial cells almost form nodules. Many neurons contain 1-6, round to oval, homogeneous intracytoplasmic inclusions with a $<2\mu$ halo (Negri bodies).

Contributor's Morphologic Diagnosis:
Medulla oblongata: nonsuppurative

meningopolioencephalitis with gliosis and intracytoplasmic inclusions (Negri bodies).

Etiology: Lyssavirus of rabies

Contributor's Comment: The histologic lesion of rabies in cattle shows a wide range of variability in the polioencephalitis.^{8,9,10} Ganglionitis was severe in this cow, and it should be remembered that rabies virus is one of several that causes ganglionitis. It has been said that inflammation in bovine rabies is minimal compared to other species, but cases seen in our area are often florid like the present case. This may reflect the fact that animals are clinically ill for at least 6 days. Early sacrifice is thought to result in milder lesions. The presence of Negri bodies is thought to be inversely related to the degree of inflammation. Regardless, the presented case had significant inflammation and widespread Negri bodies throughout the brain as well as in the Purkinje cells, a common site in cattle.



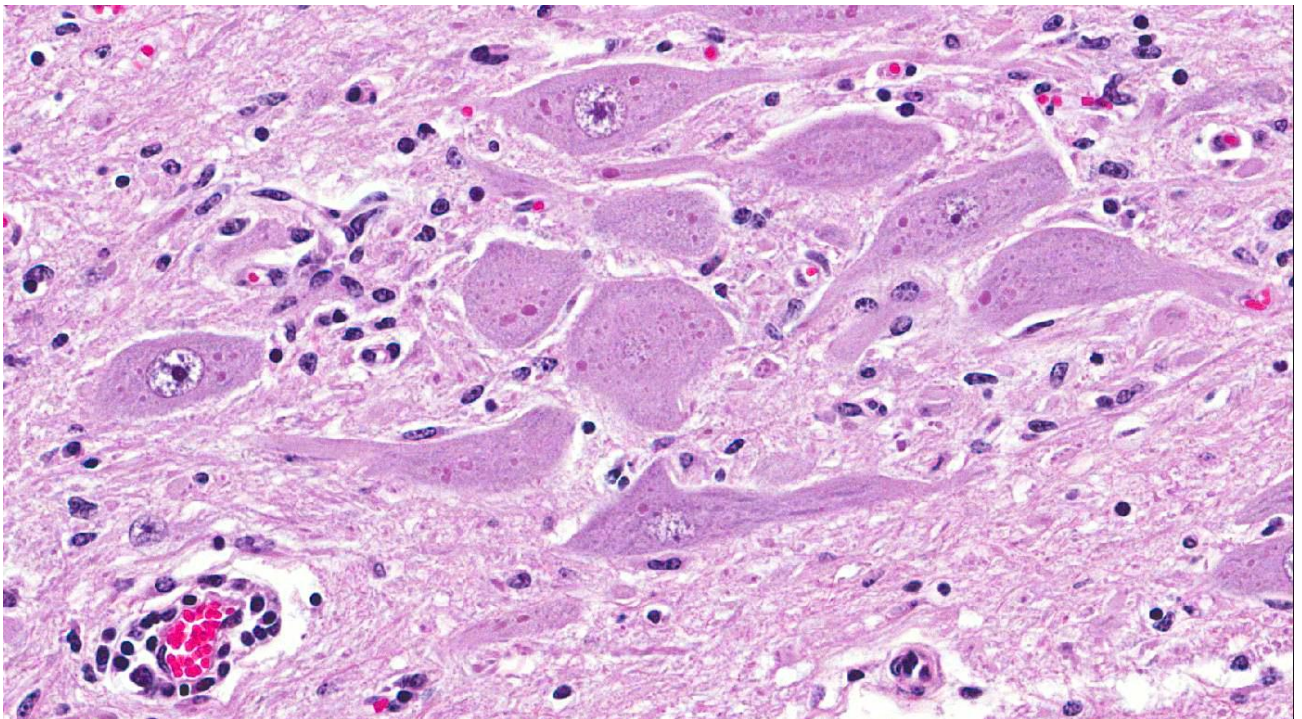
Grey matter vessels are cuffed by five to six layers of lymphocytes with diffuse gliosis of the grey matter. (HE, 80X)

Texas is setting records in rabies cases with several hundred cases reported in the counties adjacent to our laboratory. Because of baited vaccination of wildlife, we have now got less fox and coyote rabies and lots of skunk rabies.⁷ Nonhematophagous bats are also commonly infected.¹

The history was classic (6, and being forewarned, all precautions were taken in taking samples to confirm rabies, as well as completing a complete necropsy. While papers about the virtues of immunohistochemical (IHC) techniques are touted, we must conform to the use of our State's official test that is a direct immunofluorescent (DIF) test on chilled brain. That may be a good thing in view of a comparison of diagnostic tests on 26 naturally infected cows in Brazil where IHC detected only 92.4% of cases.⁶ The official sample in Texas is a transverse section of the brain extending caudally from the colliculi to the level of and

including the midcerebellar cortex. Previous sampling used a midsagittal half of the entire brain, or half of a transverse section of the brain at the level of the cerebellar roof nuclei and the hippocampus (This allowed us to store the other half of these sections in the event of loss of sam-ples.). The previous sites were based on the belief that the cerebellum was best for ruminants while the hippocampus was best for other species. Our State lab has had rare cases where the brain was positive on one side; therefore, we now include transverse pieces of entire brainstem. Otherwise, we will not get an official "negative" test. Our greatest liability challenges are not dealing with positive cases; rather, things get exciting with getting neither negative or positive results due to improper sampling or sample submission.

Historically, diagnosis of rabies has involved a variety of tests. Direct immunofluorescence, direct immunohistochemistry,



Neurons within brainstem nuclei contain numerous 2-4um brightly eosinophilic intracytoplasmic viral inclusions (Negri bodies). (HE, 360X)

mouse inoculation and the demonstration of meningoencephalitis with Negri bodies have been used. A recent comparison of techniques in diagnosis of bovine rabies supported the belief that the cerebellum was an area of 100% positivity with mouse inoculation and direct immunofluorescence; however, the pons, spinal cord and thalamus were also good. Negri bodies were seen 82% of the time in the cerebellum.⁽⁴⁾ Similar results were found in a second study.⁽²⁾ A recent immunohistochemical study of archival tissues of rabies in a variety of species concluded “the best site for rabies virus detection in dogs and cats was the hippocampus, but in cattle, viral antigen was most prominent in the brainstem, followed by the cerebellum. In horses, the cervical spinal cord and adjacent brainstem were the optimal sites for detecting rabies virus antigen. In raccoons and skunks, labeling was dispersed more widely; thus, tissue site selection might be less important for these wildlife reservoir species.”⁹

It is always difficult to compare results using different techniques conducted in different laboratories, but I hope whoever examines tongue tone in cattle showing the sign of this cow wears proper PPE!

JPC Diagnosis: Brainstem: Meningoencephalitis, nonsuppurative, diffuse, moderate, with gliosis and neuronal intracytoplasmic inclusion bodies.

Conference Comment: Conference participants described the inflammatory infiltrate as mild to moderate in severity, composed of lymphocytes, plasma cells, and macrophages, expanding Virchow-Robin spaces up to five times normal thickness, as well as being present surrounding vessels in adjacent meninges. The endothelium of affected vessels is multifocally hypertrophied, and there are prominent areas of

gliosis as described by the contributor. There was slide variation in the number of Negri bodies present, with some slides having an abundance, most prominently in brainstem nuclei.

Rabies virus testing was discussed during the conference in the context of working in areas of the world where high ambient temperatures and lack of refrigeration may present challenges that make the fluorescent antibody test less feasible, and the use of other methods such as immunohistochemistry (IHC) more practical. However, as mentioned above by the contributor, the accuracy of IHC can be less than 100%; nonetheless, some studies have shown the accuracy of IHC to be very similar to fluorescent antibody testing, indicating it may be a feasible alternative in some cases. Important considerations for IHC include the species of mammal being tested in regard to the area of brain sampled as discussed above, the type of antibody used (polyclonal vs. monoclonal) and the time the tissue remained in formalin.⁹ Reverse transcription-PCR for rabies diagnosis has been developed and has been shown to have similar results when compared with the fluorescent antibody test,³ and in some cases has been shown to be superior when there is significant decomposition in the sample.⁵

Besides more commonly implicated wildlife species such as foxes, skunks and raccoons, bats are also an important wildlife rabies reservoir, capable of transmitting the virus to domestic species and humans. Bats are one of the most commonly infected wildlife species in Texas and were indeed the most commonly infected species between 2006 and 2010.⁴ A great deal is unknown regarding the complex pathogenesis of rabies in bats, and although uncommon, adaptation of bat rabies virus variants into other mammals has occurred.⁴ Bats may be exposed to the virus

multiple times in their lives, via different routes, which can influence outcome in future exposures. Clinical outcome can vary significantly in infected bats, from rapid clinical progression to survival for several months after infection, and route of infection may play a role in how the disease progresses and the bats' ability to transmit the disease.² Rabies remains an important public health problem in the United States and worldwide; understanding testing methodology and pathogenesis, in order to facilitate prompt diagnosis in cases of human exposure and to help control infection in domestic species, remains important for public health officials and pathologists alike.

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CASE III: 30334-08 (JPC 3133960).

Signalment: 3-year-old, neutered, hermaphrodite, Pug (*Canis familiaris*)

History: The dog was seen at an animal hospital clinic on 8/8/08 with a high

temperature, anxious appearance, tense abdomen and painful in the lumbar area. CBC, Chemistry panel, urinalysis and radiographs of the spine, abdomen and thorax were within normal limits. It was treated for vertebral disk syndrome. No improvement



Dog, hypothalamus. An infiltrative neoplasm extends up into the thalamus. (HE, 4X).

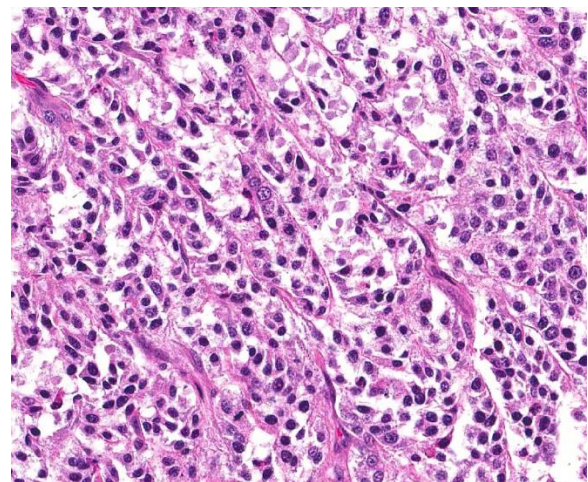
was noted after initial treatment with dexamethasone. After 12 days post presentation the dog's condition deteriorated and was hospitalized. Abnormal physical findings included anorexia, knuckling of the rear legs, circling to the left, dilated right pupil, constricted left pupil, semi consciousness, protruding tongue, lateral recumbency and lack of response to stimuli. The dog became unconscious before death.

Gross Pathology: The ventral surface of the brain has a large irregular mass destroying the pituitary gland, most of the thalamus and the optic chiasma. The mass extended along the base of the skull and measured approximately 7 X 1.5 X 3 cm. On longitudinal section, the brain has an approximately 1.3 cm in diameter, granular,

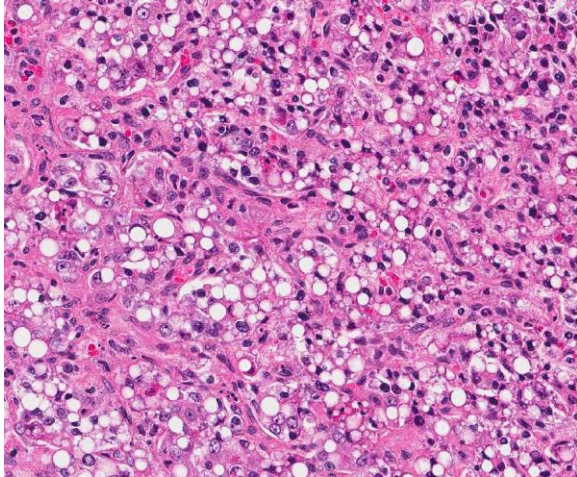
greenish colored, irregular round mass primarily within the thalamus and third ventricle.

Laboratory Results: Immunohistochemistry stains for vimentin (VM) and cytokeratin (CK) are positive. Within the neoplasm, the small cell population constantly strongly stained with CK. On the other hand, the large cell population was mostly stained with (VM). Stains for NSE, S100 and GFAP are negative.

Histopathologic Description: Brain and meninges: Within the meninges and extending into the gray and white matter is a poorly circumscribed, expansile, invasive, densely cellular neoplasm subdivided in lobules and packets by a fibrovascular stroma. The neoplasm is composed of two populations of pleomorphic cells and three patterns. One cell population consists of large polygonal cells arranged in pseudorosettes, occasionally around a central vascular core, and cords separated by fine fibrovascular stroma. These cells have distinct cell borders and moderate to abundant, wispy eosinophilic (hepatoid-like appearance), frequently vacuolated (signet ring-like appearance)



The primary cell population are small poorly differentiated polygonal germ cells with scant cytoplasm which are arranged in nests and packets. (HE, 200X)



Adjacent to nests of germ cells is a second populations of cells with prominent clear cytoplasmic vacuoles, also in nests and packets. (HE, 200X)

cytoplasm. Occasionally hepatoid type cells have apical brush borders. The nuclei are large, vesicular, irregularly round, central or peripherally located with finely stippled chromatin and one or two nucleoli. The second population of cells is smaller (round and epithelial type) and they are scattered throughout the neoplasm. These cells have scant eosinophilic cytoplasm, indistinct cell borders and round nuclei with clumped chromatin. Mitotic figures range from 3-5 per 400X field in some areas. There is marked anisocytosis and anisokaryosis, and individual cell necrosis. There are extensive areas of necrosis characterized by cellular and eosinophilic debris, mild hemorrhage, degenerate neutrophils, plasma cells and MOTT cells interspersed between neoplastic cells. Within the adjacent gray and white matter, there is multifocal moderate gliosis with occasional glial nodules, satellitosis and scattered neuronal necrosis.

Contributor's Morphologic Diagnosis: Suprasellar germ cell tumor

Contributor's Comment: Germ cells give rise to spermatogonia in the testis and oogonia in the ovary. Gonadal germ cell

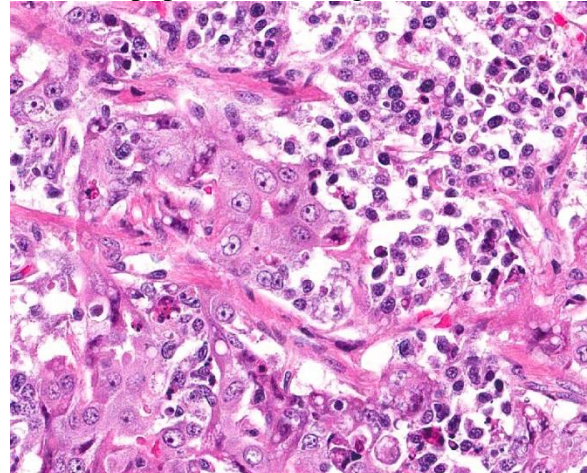
tumors more commonly reported in domestic animals include seminomas, teratomas, embryonal carcinomas, and mixed germ cell-sex cord stromal tumors. However, extragonadal tumors have been reported including the intracranial suprasellar germ cell tumor and mixed germ cell tumor in the eye and spinal cord of dogs.^{2,6} Suprasellar germ cell tumors or germinomas are very rare neoplasms in man (children and adolescents) and young dogs presumed to arise from ectopic migration of germinal epithelium from the yolk sac (embryonic stages) and its persistence at these novel sites. A recent genetic study of intracranial germ cell tumor in man suggests that these tumors are derived from cells that retain, at least partially, an embryonic stem cell-like phenotype, which is a hallmark of primordial germ cells.³ The preferred location of these tumors, as the name refers, is the pineal and hypothalamus region above the sella turcica. Microscopically, these tumors vary in cell morphology and patterns; primarily from large polygonal cells with hepatoid-like or vacuolated signet-like appearances forming cords and pseudo-rosettes (similar to hormone-producing cells or gonadal teratomas) to small cells with small amounts of cytoplasm forming nests scattered throughout the neoplasm (similar to a neuroendocrine pattern or resembling seminomas).⁸ Few foci of epithelial cells (cuboidal or squamous epithelial cells) can be seen within the tumor. Neoplastic cells are very pleomorphic making difficult to establish the difference between two or more populations of neoplastic cells. However, other authors described three populations of cells: round cells with a large round to ovoid nucleus and indistinct borders arranged in clusters, large hepatoid cells with distinct borders and compact or vacuolated cytoplasm arranged in trabeculae and epithelial cells (columnar or cuboidal, occasional squamous cell differentiation) forming tubuloacinar structures.²

Immunohistochemically, these tumors express alpha-fetoprotein, vimentin and keratin. Alpha-fetoprotein is a positive marker for germ cell tumors in humans and dogs, and is produced by yolk sac tumors, enteric elements of teratomas and some embryonal carcinomas.⁶ The diagnosis of the suprasellar germ cell tumor is based on three criteria: 1) midline suprasellar location, 2) presence within the tumor of several distinct cell types (histomorphology), and 3) positive staining for alpha-fetoprotein, VM and CK. Within the sellar region, the WHO classification of tumors from the nervous system of domestic animals has four tumors that include pituitary adenoma, pituitary carcinoma, craniopharyngioma and suprasellar germ cell tumor.⁴

The neoplasm was considered as a suprasellar germ cell tumor as a primary differential based on the pleomorphism of the neoplastic cells with different patterns and gross location. However, alpha-fetoprotein, which is an important marker for this type of tumor, was unavailable in the laboratory. In addition, this tumor was highly cellular and invasive with extensive areas of necrosis and frequent mitotic figures. Anaplastic meningioma was also considered as differential diagnosis since the microscopic location is associated with the meninges, the tumor has features of malignancy and the neoplastic cells are CK and VM positive. However, meningiomas are usually present in middle aged to older dogs. Furthermore, anaplastic astrocytoma and craniopharyngioma should also be considered since they share several histomorphological features with suprasellar germ cell tumor. Craniopharyngioma has areas resembling ameloblastoma and usually the mitotic rate is low.

JPC Diagnosis: Cerebrum: Suprasellar germ cell tumor.

Conference Comment: Overall the conference histologic description was similar to the contributor's description. Many thought there were only two populations of cells, but some participants described three: 1) polygonal cells arranged in tubules, acini and/or rosettes, 2) polygonal cells arranged in small islands and cords, and 3) round to polygonal cells arranged in loose sheets, with the third population having multifocal areas

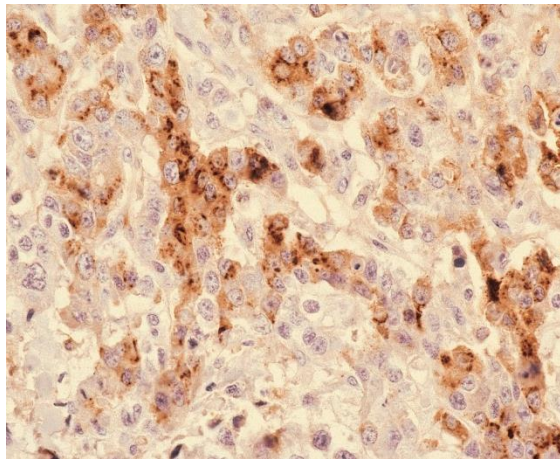


A third population of polygonal epithelial-like cells is scattered throughout the mass. (HE, 200X)

of individual cell necrosis. The different cell populations were described as being haphazardly intermingled, making specific patterns difficult to discern. There was discussion regarding the presence of “epithelial-like” cells, though some participants thought this population was not a prominent feature of the neoplasm. Participants also described deeply basophilic amorphous material and focally extensive areas of lytic necrosis. Immunohistochemical stains evaluated included alpha-fetoprotein, vimentin and pancytokeratin; there was multifocal cytoplasmic immunoreactivity in each with the strongest positivity demonstrated with pancytokeratin.

The differential diagnosis list for neoplasms in the suprasellar region were discussed including craniopharyngioma. This tumor arises from remnants of Rathke's pouch.

Craniopharyngiomas are composed of polygonal to columnar cells arranged in solidly cellular areas but may also be seen arranged in cysts or tubules. These tumors generally have areas of squamous differentiation and ciliated cells lining cystic spaces, neither of which were present in the tumor in this case. They can have multifocal areas of necrosis, and cholesterol crystals may be seen. Pituitary adenoma and pituitary carcinoma were also discussed. Pituitary adenomas are composed of polygonal to spindle shaped cells which can be arranged in solidly cellular areas as well as a sinusoidal pattern, and they lack the characteristic of multiple cell types as seen in the tumor in this case. The nuclei in pituitary adenomas are vesiculate, similar to the tumor in this case, but the cells have a moderate to abundant amount of granular cytoplasm. Pituitary carcinomas are similar to adenomas but are more invasive with greater cellular atypia and higher mitotic rate.⁴



Cytoplasm of the epithelial-like cells strongly positive for alpha fetoprotein. (anti-AFP, 200X)

Grossly, suprasellar germ cell tumors are grey-white in color, located on midline and usually obscure the pituitary and compress overlying neuroparenchyma. Pituitary tumors are white to brown in color and can be quite large, also compressing the adjacent neuroparenchyma.⁴ Craniopharyngiomas are

also large tumors that grow along the ventral brain, but may extend dorsally into the neuroparenchyma.⁵ Each of these is a reasonable gross differential diagnosis for a mass located in the most ventral region of the brain, on midline and caudal to the optic chiasm.

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CASE IV: N 1241 (JPC 4067882).

Signalment: Thirteen-month-old male neutered orange roan English Cocker Spaniel dog (*Canis familiaris*) weighing 9.1 kg.

History: One month history of weight loss, severe azotemia [creatinine 740 mg/dL (0.3-1.7 mg/dL)] and marked proteinuria [urine protein to creatinine ratio UP:UC of 22 (<0.5)]. Lethargy and anorexia. Euthanasia.

Gross Pathology: The animal presented 24 hours after euthanasia and showed a relatively poor body condition. There was a moderate subcutaneous edema mainly affecting the ventral abdominal and inguinal area. Approximately 150 ml of yellow-red transparent liquid was found within the abdomen (ascites) and 100 ml of similar fluid within the thorax (hydrothorax). The lungs were moderately oedematous. Both kidneys were diffusely pale tan and presented with a diffusely rough and granular appearing surface with several multifocal small (up to 2 x 1 cm) pitted. On incision, the cortex

showed a generalized punctiform or granular appearance.

Laboratory Results:

DNA was extracted from hair follicles collected at necropsy. PCR amplifying exon 3 of gene *COL4A4* for detecting a single nucleotide substitution (A → T) at the base 115 (1) showed that the animal was a mutated homozygous at this position (PCR performed by ANTAGENE, France).

Histopathologic Description: Kidney: All glomeruli show a marked segmental to global damage accompanied by a mild to moderate multifocal interstitial inflammation and fibrosis. Most of the glomeruli contain abundant brightly eosinophilic extracellular homogenous (proteinaceous) material, which appears to be mainly within the Bowman's space. This proteinaceous material is stained bright red with Masson's trichrome, purple with Periodic acid Schiff (PAS) and unstained with Congo red stain. Often, glomeruli show multifocal to diffuse thickening of the Bowman's capsule, segmental to global thickening of capillary basement membranes and mild to moderate increase in cellularity. Adhesions between glomerular tuft and Bowman's capsule (synechia) and periglomerular fibrosis are common. Some glomeruli are sclerotic and obsolescent. There is a mild to moderate interstitial, often periglomerular, lymphoplasmacytic inflammation, and mild to moderate interstitial fibrosis which often extends from the cortex to the medulla forming radial streaks. Tubules are frequently mildly to moderately distended with intraluminal eosinophilic extracellular homogenous material (protein) and scant cellular debris and lined by flattened epithelial cells. Within the cortex, the interstitium appears moderately hypercellular (suspected reactive fibroblasts and/or immature mesenchymal cells).



Kidneys, dog. Both kidneys are very pale and show a diffusely rough and granular appearing surface and cortex with moderate multifocal to coalescing pitting. (Photo courtesy of: School of Veterinary Medicine and Science, University of Nottingham, UK <https://www.nottingham.ac.uk/vet/servicesfortheveterinaryprofession/pathology.aspx>)

Contributor’s Morphologic Diagnosis:

Kidney: Severe, generalized, segmental to global, chronic membranoproliferative glomerulonephritis with glomerulosclerosis and proteinuria.

Condition: Hereditary nephropathy / Familial nephropathy

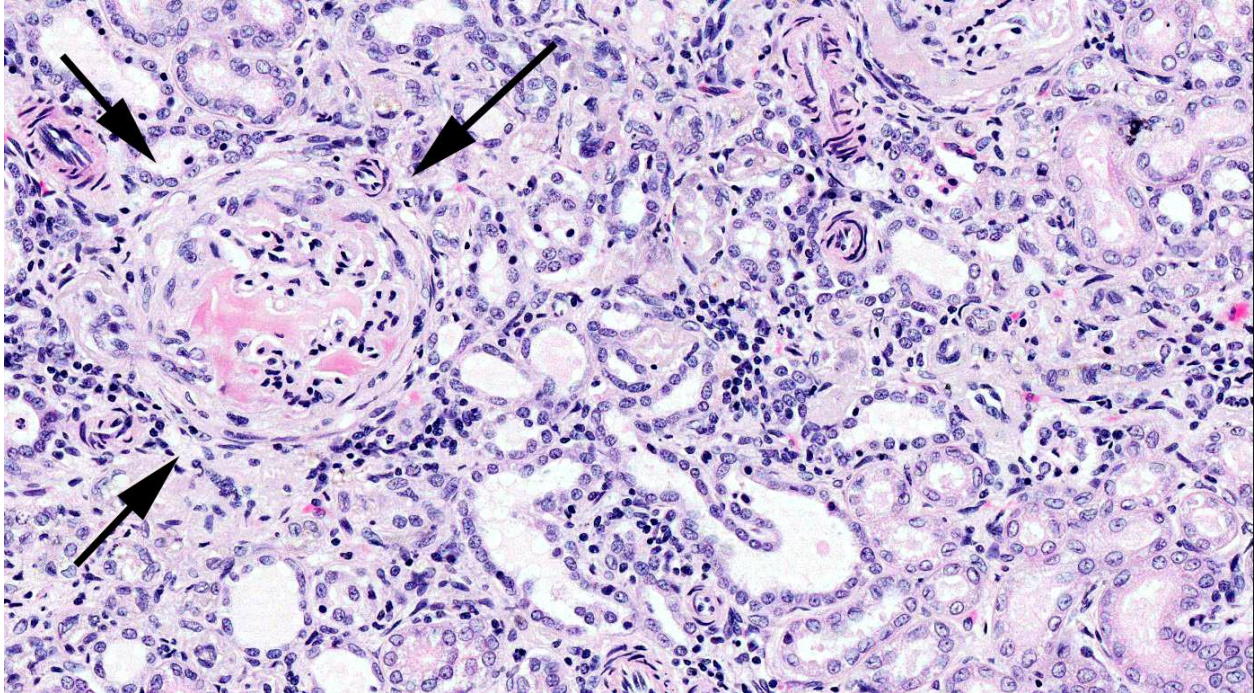


1Kidney, dog. The kidney has multifocal areas of pallor and an undulant capsular surface (HE, 5X).

Contributor’s Comment: The clinical presentation and the lesions observed in the kidneys indicated a renal failure. The edema

observed in multiple organs and body cavities was very likely secondary to the proteinuria.

Hereditary nephropathy (HN) (also called Familial Nephropathy or Hereditary Nephritis) is the most commonly used name for kidney diseases that occur in dogs due to genetic type IV collagen abnormalities.^{2,7,10} This group of diseases is considered analogous to human Alport nephropathy, although the latter presents usually with ocular and/or hearing abnormalities which have not been reported in dogs.^{9,3-6} To date, 4 different collagen IV gene mutations have been identified in dogs with HN (table 1). HN in the English Cocker Spaniel is an invariably progressive and ultimately fatal renal disease, which typically causes renal failure between 6 months and 2 years of age. The disease is inherited as an autosomal recessive trait and based on a genetic defect caused by a single base substitution in the exon 3 of *COL4A4*



Kidney, dog. There are changes at all levels of the nephron. Glomeruli (arrows) are hypercellular with synechiae formation, abundant protein within Bowman's space, and marked periglomerular fibrosis. Tubules are decreased in number, ectatic, and often lined by attenuated cuboidal epithelium. The interstitium is expanded and infiltrated by moderate numbers of lymphocytes and plasma cells. (HE, 60X).

gene (A → T).² Collagen (type IV) defects cause severe structural and functional alterations in glomerular basement membranes (GBM).^{7,4,5} Unlike most collagens, type IV collagen occurs only in the basement membranes (BM) and comprises up to six genetically distinct α -chains (designated as $\alpha 1(IV)$ to $\alpha 6(IV)$) encoded by 6 genes (*COL4A1* to *COL4A6*).⁶ Three defined trimer combinations ($\alpha 1\alpha 1\alpha 2$, $\alpha 3\alpha 4\alpha 5$ and $\alpha 5\alpha 5\alpha 6$) are formed from the six $\alpha(IV)$ chains.⁵⁻⁷ The $\alpha 1$ and $\alpha 2$ chains are present in the BM of all tissues, whereas the other four chains have restricted tissue distribution during the development.^{5,6} The expression of collagen IV chains is also subjected to temporal regulation.⁶ In the GBM, genes encoding the $\alpha 1$ and $\alpha 2$ chains are expressed during embryonic development, but their levels gradually decrease as the expression of genes encoding the $\alpha 3$, $\alpha 4$, and $\alpha 5$ chains starts. This switch explains why the affected animals are born apparently

healthy and have an early onset of disease. In affected English Cocker Spaniel dogs, the mutation in *COL4A4* prevents a normal synthesis of $\alpha 4$ chains and therefore inhibits a normal assembly of $\alpha 3\alpha 4\alpha 5$ collagen type IV heterotrimers in mature glomeruli. Instead, these heterotrimeric units are replaced by others lacking $\alpha 4$, which are suggested to be structurally weaker or less resistant to proteolytic degradation. These structural changes in the GBM lead to glomerular damage due to abnormal cell-cell and matrix-cell interactions, and a progressive tubule-Interstitial injury.⁷

Affected English Cocker Spaniel puppies typically present with proteinuria as their first clinical manifestation of the disease at the age of 2 to 8 months. Renal damage progresses and end-stage renal disease is often present at 12 months of age (ranging from 6 to 18 months), as was the case in the dog here presented.⁷

Breed	Mode of inheritance	Gene affected	Location within gene	Specific mutation	Reference
Samoyed	X-Linked	COL4A5	Exon 35	Single nucleotide substitution (G→T)	9
Mixed breed from Navasota, Texas (USA)	X-Linked	COL4A5	Exon 9	10-base pairs deletion	10
English Cocker Spaniel	Autosomal recessive	COL4A4	Exon 3	Single nucleotide substitution (A→T)	1
English Springer Spaniel	Autosomal recessive	COL4A4	Exon 30	Single nucleotide substitution (C→T)	11

Table 1. Genetic collagen IV defects identified in dogs (adapted from Ref. 2).

Contributors were unable to investigate a possible familiar occurrence of renal disease in the kindred of the dog presented here, since no information about it could be obtained.

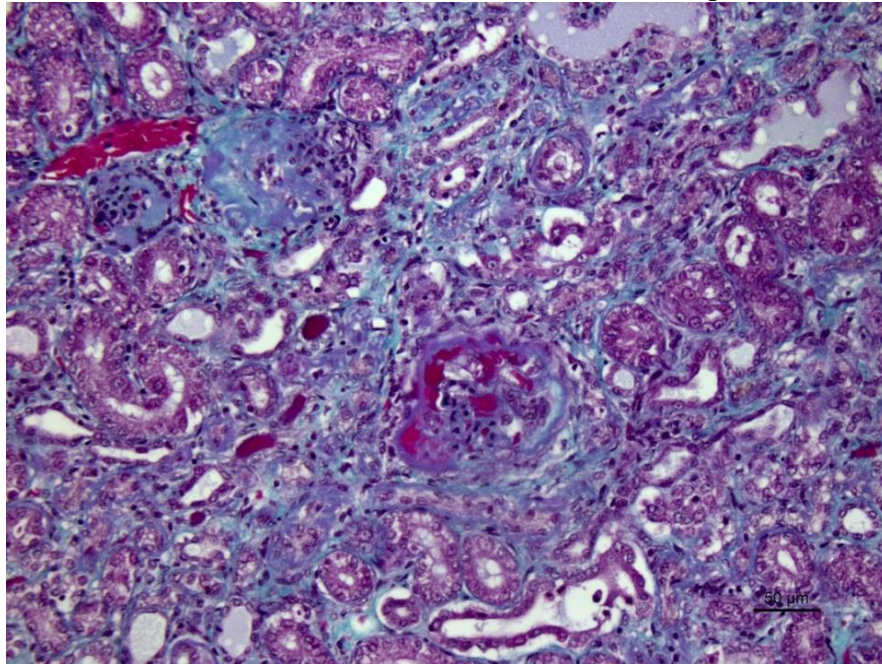
The light microscopic lesions in the kidney of HN-affected dogs are reported to be segmental thickening of GBMs and mesangial expansion, progressing through glomerular and periglomerular fibrosis and glomerulosclerosis. Usually, these lesions are accompanied by tubulointerstitial nephritis, interstitial fibrosis and tubular atrophy.^{7,10} All these light histological features were observed in the present case. The striking presence of extracellular material observed within the Bowman's spaces of most glomeruli is unusual in the contributors' opinion. Based on its appearance in hematoxylin and eosin (HE) and the performed special stains, the contributors interpret this material as a very protein-rich glomerular filtrate.

Immunohistochemistry (IHC) and transmission electron microscopy (TEM) are useful techniques to further characterize the lesion,

but they were not performed in the present case. Normal mature GBM is known to strongly express $\alpha 3$, $\alpha 4$ and $\alpha 5$ chains of type IV collagen. In English Cocker Spaniels with HN, however, the expression of those chains is either absent ($\alpha 4$) or greatly reduced ($\alpha 5$). Additionally, there is increased expression of three other chains ($\alpha 1$, $\alpha 2$, and $\alpha 6$) that would normally only be minor components of the mature GBM and therefore weakly expressed.⁷ Ultrastructural GBM changes are common to all the genetically characterized examples of HN. The principal change is an irregular thinning or thickening of GBM with splitting and fragmentation of its lamina densa. There is also often fusion of visceral epithelial cell foot processes and wrinkling of glomerular capillary walls.^{7,8} In this case, the definite diagnosis was based on a DNA test offered by ANTAGENE in France. Genetic tests are recommended in cases of suspected HN in English Cocker Spaniels and are widely available (OptiGen, Laboklin, Genetic Technologies, ANTAGENE, Genindexe, Genomia, Van Haeringen)

(www.thekennelclub.org.uk/media/14688/dn)

[atestsworldwide.pdf#sthash.eQZ2iMgB.dpu](#)
f).



Kidney, dog. There is moderate interstitial, periglomerular and glomerular fibrosis (stained in blue). The extracellular (proteinaceous) material present within glomerular Bowman's space stains bright red. Masson's trichrome, 200X). (Photo courtesy of: School of Veterinary Medicine and Science, University of Nottingham, UK <https://www.nottingham.ac.uk/vet/servicesfortheveterinaryprofession/pathology.aspx>)

JPC Diagnosis: Kidney: Membrano-proliferative glomerulonephritis, diffuse, marked with tubular atrophy and loss, and interstitial nephritis with fibrosis.

Conference Comment: The conference histologic description was very similar to the contributor's description. However, without knowledge of the signalment, history, or access to special stains, nearly all participants placed amyloidosis at the top of the differential diagnosis. Indeed, the histologic features of this entity when viewed only on an HE-stained section share many features with amyloidosis. The microscopic findings in cases of hereditary nephropathy (HN) are indicative of glomerular disease, but are not specific for or unique to HN.⁷ In addition to the above changes, moderate numbers of glomeruli were described as undergoing

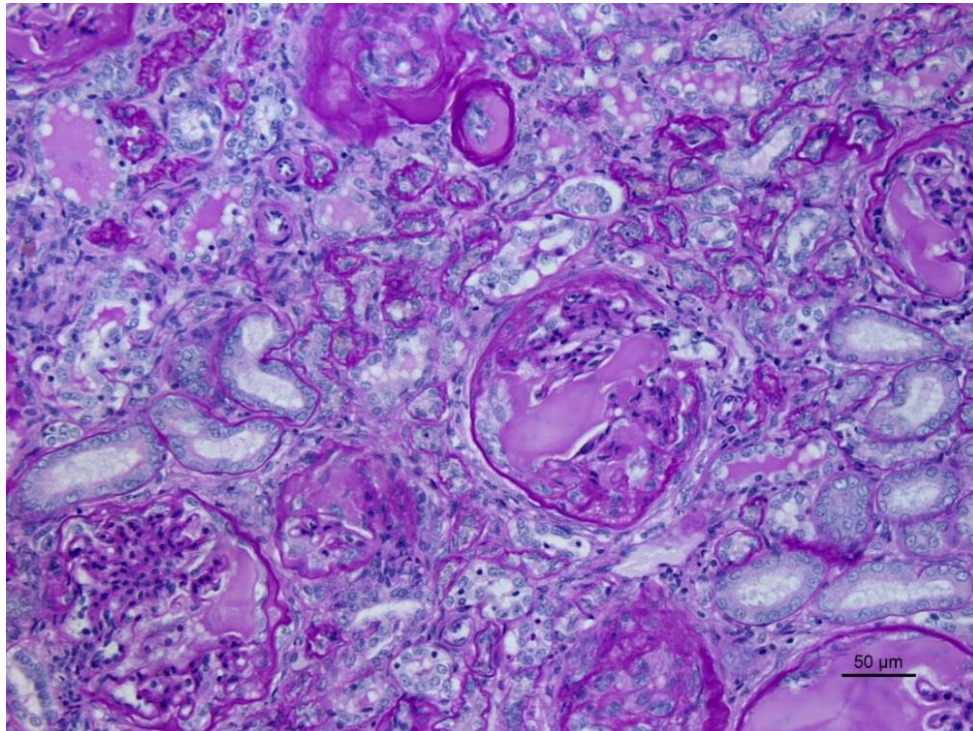
cystic glomerular atrophy with a characteristic small glomerular tuft and expanded Bowman's space.

Nonspecific changes which occur in the tubules and interstitium in glomerular disease include tubular degeneration, atrophy, interstitial fibrosis and nephritis, as was described in this case. These secondary changes tend to increase in severity as the primary glomerular disease progresses. Although the exact mechanism resulting in the tubular and interstitial changes in this condition is not completely understood, the underlying process is presumed to be similar for the development of tubular lesions in other

glomerular disorders.⁷ Blood is supplied to the tubules via the vasa recta from the efferent glomerular arteriole; in cases of glomerulosclerosis, this blood flow through the vasa recta is decreased, resulting in hypoxia and subsequent tubular epithelial cell death. Damaged tubules thereupon become lined by cuboidal or squamous cells which do not have a brush border and lack normal tubule cell function. Chronic proteinuria is also reported to damage tubular epithelium.¹¹

In the initial stages of HN, biopsies from affected dogs presenting with proteinuria can be normal. Early histologic changes include mild focal and segmental mesangial expansion, and the lesions eventually progress to global glomerulosclerosis as described above. The distinctive features of

this entity are demonstrated via electron microscopy, as well as with IHC that is specific for the different types of type IV collagen alpha chains. Tissue IHC is necessary to demonstrate the presence of the abnormal collagen chains because not all dogs that have ultrastructural changes consistent with HN have evidence of abnormal type IV collagen. Although proteinuria is a classic clinical manifestation of this condition and moderate hypoalbuminemia is seen, affected animals usually do



Kidney, dog. There is moderate thickening of the Bowman's capsule and basement membranes of glomerular capillaries and renal tubules (stained purple). The extracellular (proteinaceous) material present within the Bowman's space stains purple. (Periodic acid Schiff, 200X) (Photo courtesy of: School of Veterinary Medicine and Science, University of Nottingham, UK <https://www.nottingham.ac.uk/vet/servicesfortheveterinaryprofession/pathology.aspx>)

not have nephrotic syndrome or hypertension, but rather develop a worsening uremia as the condition progresses.⁷

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