



WEDNESDAY SLIDE CONFERENCE 2014-2015

Conference 4

24 September 2014

**CASE I:** 13110510 (JPC 4048566).

**Signalment:** Tissue from an 8-year-old intact female Miniature Rex rabbit (*Oryctolagus cuniculus*).

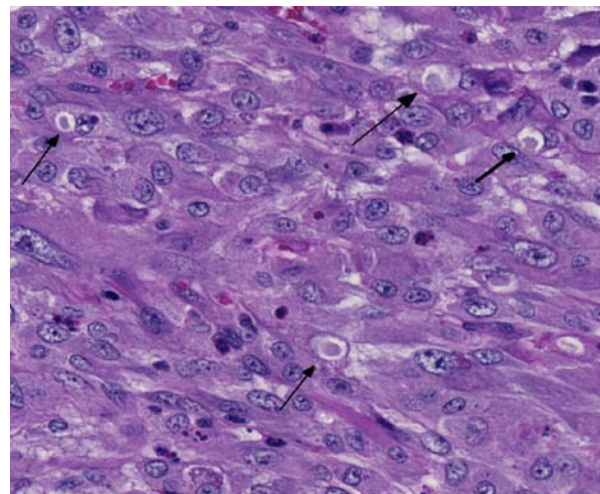
**History:** Tissue samples were received on routine biopsy service. History submitted by referring veterinarian indicated relatively acute onset of multiple cutaneous masses distributed “all over” patient’s body.

[Follow-up by phone conversation: The referring veterinarian was contacted by phone 6 months after biopsy submission. The veterinarian reported that all masses that had abruptly appeared regressed and were completely gone by 2-3 months. The veterinarian reported that the rabbit was currently healthy.]

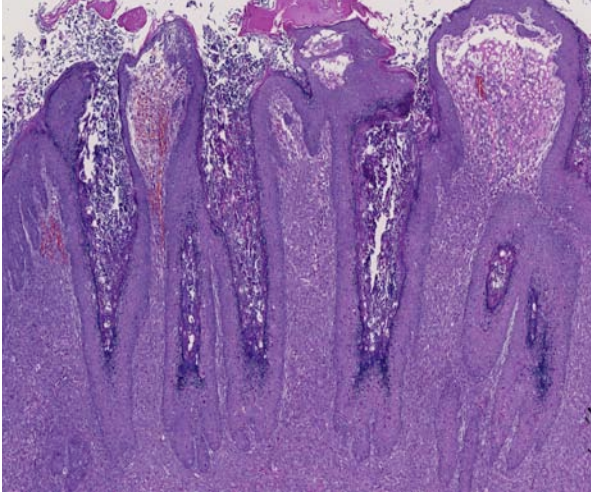
**Gross Pathology:** Two biopsies of consisting of haired skin were received. Each contained a nodular, well-demarcated firm mass that was solid



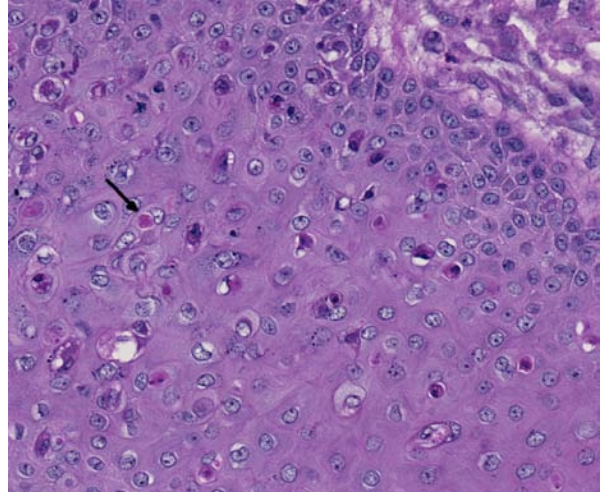
1-1. Haired skin, rabbit: The dermis is expanded by a densely cellular neoplasm which elevates the overlying hyperplastic epithelium. (HE 0.63X)



1-2. Haired skin, rabbit: The neoplasm within the dermis is composed of densely packed hypertrophic fibroblasts which occasionally contain intracytoplasmic poxviral inclusions. (HE 256X)



1-3. Haired skin, rabbit: The epithelium overlying the neoplasm is markedly hyperplastic with abundant hyperkeratosis. (HE 35X)



1-4. Haired skin, rabbit: The hyperplastic epithelium contains ballooning degeneration and necrosis of individual hepatocytes as well as intracytoplasmic poxviral inclusions. (HE 256X)

and homogeneous white/tan on cross section. The masses were 2.2cm and 3cm in diameter.

**Laboratory Results:** Not provided.

**Histopathologic Description:** Tissue samples are from haired skin or skin from mucocutaneous junction. Lesions are similar in each. The dermis is expanded by a raised, well-demarcated, non-encapsulated neoplastic mass. The mass is comprised of densely-cellular, primarily spindled to polyhedral cells arranged in streams or short, haphazardly-placed bundles or fascicles. The neoplastic cells have large nuclei exhibiting moderate anisokaryosis and prominent, often multiple, nucleoli. The cells have moderate amounts of amphophilic cytoplasm with rare, intracytoplasmic, round, eosinophilic inclusion bodies. There are 2 mitotic figures seen in 10 random high power fields examined. The epidermis overlying the mass is variably hyperplastic with regional orthokeratosis and occasional serocellular crusts. Innumerable epidermal cells at nearly all levels (strata) are rounded with either grey-smudged or vacuolated cytoplasm that includes single, conspicuous, roughly round, 5-12  $\mu\text{m}$  in diameter, bright eosinophilic inclusion bodies. Inconsistent lesions across sections include regional edema, acute necrosis of individual neoplastic cells, scattered and variably intense heterophilic infiltrates and surface ulceration.

**Contributor's Morphologic Diagnosis:** Haired skin and mucocutaneous junctions, multiple sites:

Fibromatosis with intracytoplasmic inclusion bodies.

**Contributor's Comment:** Shope fibroma virus (SFV) belongs to the *Leporipoxvirus* genus of the *Poxviridae* family of viruses. The natural host is the Eastern cottontail (*Sylvilagus floridanus*) rabbit; however, this is expanded to also include other rabbits, hares and squirrels.<sup>5,12</sup> The SFV genome has been completely sequenced and compared with the myxoma virus (MYX), which is a closely related virus that causes myxomatosis in rabbits.<sup>12</sup> These viruses are similar enough that live-attenuated SFV is administered as a vaccine providing cross-immunity towards myxomatosis.<sup>5</sup>

The lesions caused by SFV in rabbits were first described by Dr. R.E. Shope, a physician and virologist, in 1932.<sup>8,9</sup> SFV is transmitted to rabbits through biting insect and arthropod vectors such as mosquitoes and fleas.<sup>1,5</sup> Tumors can arise from each bite site, resulting in one to several masses appearing on the patient.<sup>5</sup> Lesions are most often located on the head and legs.<sup>5</sup> The disease exhibits seasonality with most cases reported in the fall (September-November).<sup>5</sup> Lesions typically reach maximum size between 7-12 days after inoculation, which can account for the relatively rapid onset noticed by owners as in this case. Afterwards, lesions will regress over 1-1.5 months leaving the host immune.<sup>5</sup> Infection by SFV in neonatal or very young rabbits can lead to fatal infections with disease resembling myxomatosis.<sup>1,5</sup>

Von Bomhard et al. performed a retrospective study on cutaneous lesions in rabbits, separating lesions into virus-induced and non-virus-induced tumors.<sup>11</sup> Shope fibroma was the most common virally induced tumor followed by Shope papilloma (*Papovavirus*). Overall, trichoblastomas and collagenous hamartomas were the most common cutaneous tumors reported from this study. Interestingly, this study reported a sex predilection for mesenchymal tumors in rabbits, with mesenchymal proliferations occurring significantly more in male rabbits than females.

**JPC Diagnosis:** Haired skin: Atypical fibroblastic proliferation.

**Conference Comment:** This case presents a beautiful example of the characteristic poxvirus intracytoplasmic inclusions and delivers an opportunity for some introspective criticism and a revisionist morphologic diagnosis by the JPC. Historically, we have favored the diagnosis of “atypical mesenchymal proliferation” for Shope fibromas and myxomas, both the result of *Leporipoxvirus* infection in rabbits. While technically accurate, it fails to identify fibroblasts as the cellular origin which is well described in multiple sources and implied within its common name “Shope fibroma”.<sup>3,6</sup>

The fibroblast proliferation is unusual among the poxviruses, which are generally epitheliotropic in nature. Viral replication within epithelial cells causes “ballooning degeneration” and necrosis, with sites of replication observable histologically as small, basophilic, intracytoplasmic inclusions designated as *type B* inclusions or Guarnieri bodies, while the larger eosinophilic inclusions as observed in this rabbit are those of *type A* which occur later in the replication cycle.<sup>7</sup>

Specific to the members of the *Leporipoxvirus* family (Shope fibroma virus, squirrel fibroma virus and myxoma virus), the proliferation of fibroblasts expanding the dermis is the more prominent histologic lesion. In SFV, poxviral inclusions are also found in these mesenchymal cells as observed in this case. In contrast, myxoma virus infections induce a more mucinous matrix separating fibroblasts which lack viral inclusions (but are seen in overlying epithelial cells).<sup>6</sup> Poxviruses are known to express proteins with similar mechanisms of action as epidermal

growth factor<sup>2</sup>, and it is logical to conclude members of the *Leporipoxvirus* family may similarly produce fibroblast growth factors, though this has not been demonstrated as research on these niche viruses is limited.

Through the discovery that pretreatment with RANTES (CCL5) to cells in culture could inhibit infection of myxoma virus, researchers uncovered its affinity for the chemokine receptor CCR5, in addition to CCR1 and CXCR4.<sup>4</sup> This is a noteworthy feature of HIV and SIV infections, in which individuals have been identified with a natural resistance to infection due to a mutation in the CCR5 gene.<sup>10</sup> These chemokine receptors are specific to white blood cells, which alludes to the immunosuppressive nature of HIV infections.<sup>9</sup> What this means, if anything, for poxviruses and specifically the behavior of the *Leporipoxvirus* family is still undetermined.

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**CASE II: WSC #1 (JPC 4048439).**

**Signalment:** <1 month-old, female white-tailed deer fawn, *Odocoileus virginianus*.

**History:** This young fawn was found weak and emaciated in the summer on a farm in Connecticut. Due to concerns about the appearance of its skin, it was shot and submitted for necropsy.

**Gross Pathology:** The fawn was in poor body condition with absence of subcutaneous and visceral fat stores. There were extensive areas of alopecia and multiple red dermal abrasions on all four legs. Innumerable, firm, 1-2 mm thick, brown crusts that entrapped hair across multiple follicles were disseminated on the skin surface of the entire body, particularly on the dorsum and dorsolateral aspects of the trunk and on the face.

**Laboratory Results:** None.

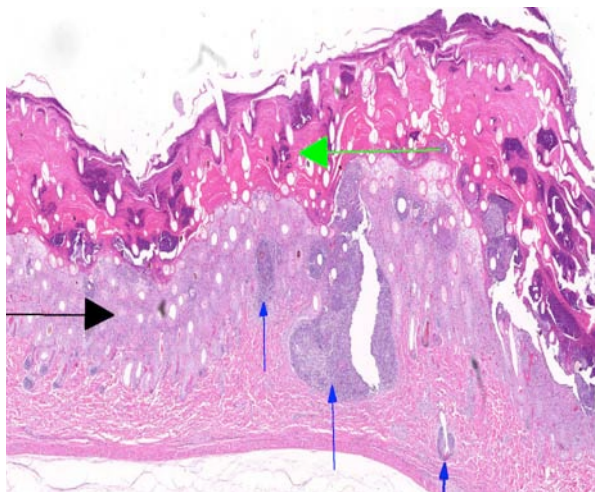
**Histopathologic Description:** Haired skin: Covering an epidermis that is frequently ulcerated are thick crusts spanning multiple hair follicles and composed of layers of keratin with retained nuclei (parakeratosis), abundant hyaline eosinophilic (proteinaceous) material, accumulations of degenerate neutrophils (intracorneal pustules) and necrotic debris, entrapped hairs and small areas of hemorrhage. Throughout the crusts and extending into inflamed and frequently ruptured hair follicles

(furunculosis) are innumerable tangled, branching, 1-2  $\mu\text{m}$  wide filamentous structures composed of parallel rows of coccoid bodies. More amorphous colonies of coccoid bacteria are also present. In the remaining epidermis there is mild diffuse acanthosis and areas of spongiosis that also involve the follicular epithelium. Large numbers of neutrophils fill follicular lumina, disrupt follicular epithelium and extend into the surrounding dermis from areas of ulceration and furunculosis. Also present in the dermis are smaller numbers of macrophages, lymphocytes and plasma cells.

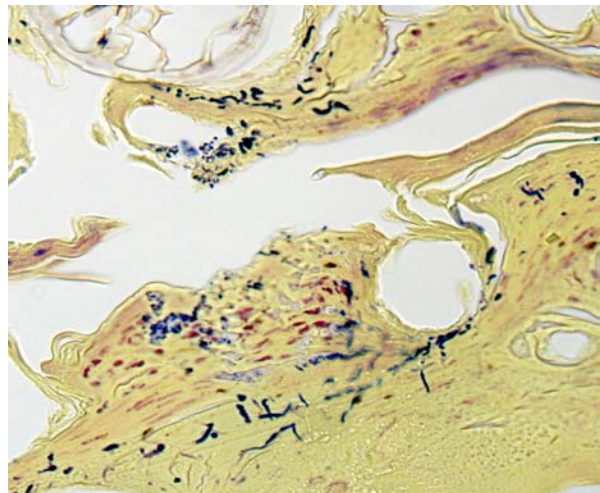
Gram Stain (not submitted): The filamentous and coccoid forms of bacteria stain gram-positive.

**Contributor's Morphologic Diagnosis:** Skin: Severe, exudative, crusting and neutrophilic dermatitis and folliculitis with ulceration, furunculosis, parakeratosis, and filamentous and coccoid bacteria, consistent with *Dermatophilus congolensis*.

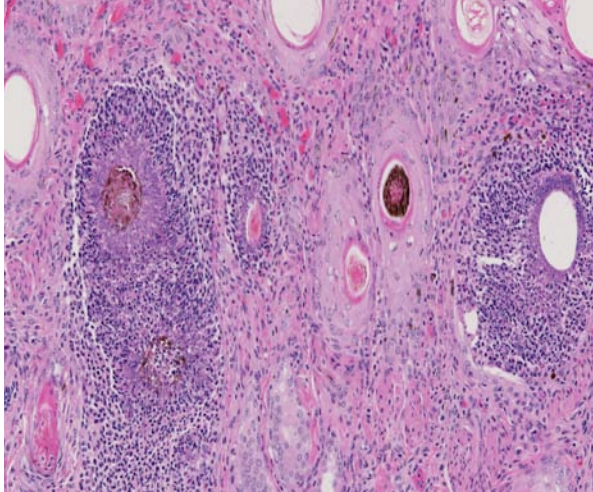
**Contributor's Comment:** The gross appearance and histopathologic findings in this deer fawn were typical of dermatophilosis, caused by the Gram-positive actinomycete *Dermatophilus congolensis*. Although *D. congolensis* has a worldwide distribution, disease has historically been more common in tropical and subtropical climates, especially during prolonged periods of rain.<sup>2,5</sup> Dermatophilosis, also termed cutaneous



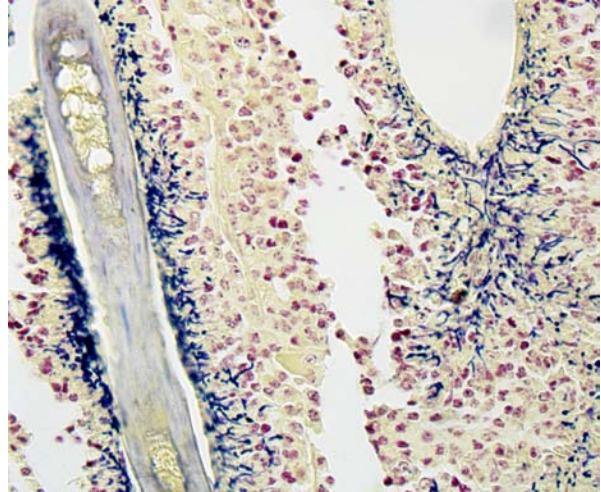
2-1. Haired skin, fawn: The epidermis is covered by a lamellated serocellular crust with numerous collections of degenerate neutrophils (pustules - green arrow). The subjacent epidermis is multifocally hyperplastic (black arrow), numerous hair follicles are surrounded or effaced by aggregates of degenerate neutrophils (furunculosis - blue arrows). (HE 6.3X)



2-2. Haired skin, fawn: The serocellular crust contain numerous filamentous chains of gram-positive zoospores, consistent with *Dermatophilus congolensis*. (Brown-Hopps 320X)



2-3. Haired skin, fawn: Hair follicles are multifocally effaced by numerous neutrophils and fewer histiocytes infiltrating along the external root sheath. (HE 280X)



2-4. Haired skin, fawn: Hair follicles contain numerous radiating filamentous chains of gram-positive zoospores, consistent with *Dermatophilus congolensis*. (Brown-Hopps 400X)

streptothricosis, cutaneous actinomycosis, rain scald, rain rot, lumpy wool, and strawberry foot rot, has been reported in many species. Among domestic animals, it is most commonly seen in cattle, sheep, and goats, with horses less frequently affected.<sup>1-3</sup> The disease is also zoonotic, and spread from white-tailed deer to humans has been described.<sup>3</sup>

The first reports in white-tailed deer were from the New York state region in the 1970s, along with an isolated case in a fawn from South Carolina.<sup>3,5</sup> A recent survey of bacterial and parasitic dermatologic diseases in white-tailed deer from 1975-2012 found a 0.7% incidence (19/2569) of dermatophilosis.<sup>4</sup> At 21.6% of the cases, this was second only to demodicosis in frequency of bacterial or parasitic skin disease among deer in the Southeastern United States.<sup>4</sup> As in initial reports in twin fawns,<sup>5</sup> lesions most commonly involved the head/face and limbs and consisted of exudation and crusting, alopecia, ulcers and erosions.<sup>4</sup> In the current case, the lesions were particularly severe on the face but were also generalized. It has been hypothesized that a predilection for the rostrum in fawns could be due to transmission from an infected dam during nursing.<sup>5</sup> Dermatophilosis has been found in fawns as young as 2 weeks old,<sup>5</sup> and the study by Nemeth et al. found significantly more juveniles affected (63%) than adults.<sup>4</sup> Debilitation and emaciation in the fawn in this case were attributed to the severity of the dermatophilosis, which has been reported as a cause of death in other affected fawns.<sup>3,4</sup>

The diagnosis of dermatophilosis in this case was based on the presence of thick lamellated crusts in association with characteristic filaments of parallel rows of Gram-positive coccoid bodies (“railroad track” configuration) formed from transverse and longitudinal septation.<sup>2</sup> A combination of skin damage and prolonged moisture are generally required for establishment and spread of infection, as the organism is minimally invasive in intact epidermis, and wet conditions are needed for activation of coccoid bodies into motile zoospores.<sup>2</sup> Ectoparasites can be a cause of trauma and may also act as mechanical vectors to spread the infection.<sup>2</sup> Although usually confined to the epidermis and outer root sheaths of hair follicles, there are rare reports of deeper skin or lymph node involvement.<sup>1,2</sup> Ulceration and furunculosis were prominent features in the current case, but there was no evidence of spread to lymph nodes or other tissues.

**JPC Diagnosis:** Haired skin: Epidermitis and folliculitis, suppurative, diffuse, severe, with epithelial hyperplasia, parakeratotic hyperkeratosis, and numerous filamentous bacteria.

**Conference Comment:** The contributor gives an exceptional overview of dermatophilosis, a distinctive disease most often observed in production animals. Buried within the pathogenesis of infection with this gram-positive, actinomycete bacterium is a glimpse of the skin’s effectiveness in providing a barrier to disease. The

combination of hair, surface lipid, and stratum corneum equate to an insurmountable barricade for *Dermatophilus* in the normal state. However, with even minor disruptions of the host's defenses combined with the moist conditions required to activate the zoospores, the bacteria incite a fulminant infection which contributes to significant meat, milk and wool production loss throughout the world.<sup>2</sup>

*Dermatophilus* produces exoenzymes and has an affinity for a low carbon dioxide environment which facilitates its colonization and tissue-specificity for the epidermis. The host responds to infection with cornification of keratinocytes and invasion of neutrophils effectively walling off the bacteria from the dermis. The progenitor cells of the follicular epithelium then propagate, forming a new layer of epidermis.<sup>2</sup> Subsequent invasion of this new layer followed by repetitive and unsuccessful defense responses of the host creates the thick laminar and parakeratotic crusts with entrapped hair follicles which are so characteristic to this bacterial infection and nicely exemplified in this case.

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**CASE III:** 09-163 (JPC 4017918).

**Signalment:** 2-year-old male Labrador retriever dog weighing 16 kg (*Canis familiaris*).

**History:** This patient was one of two Labrador retriever dogs from the same litter that were noted to have persistently pale mucous membranes and a grade I/VI systolic heart murmur. Serial complete blood counts revealed a chronic, moderate to severe, macrocytic, and strongly regenerative anemia. Extensive diagnostic workup to rule out conditions leading to a strongly regenerative anemia was performed, including those causing acquired intermittent or chronic hemolytic anemia (immune-mediated, infectious, toxic), or blood loss anemia, all of which were negative. Additional studies led to the diagnosis of hereditary erythrocytic pyruvate kinase (PK) deficiency.

Over the next several months this dog developed mildly icteric mucous membranes with progression of the heart murmur to IV/VI, although otherwise remained clinically well despite persistent anemia and few crises. Three days prior to death he became icteric and lethargic and started vomiting. His clinical condition deteriorated rapidly, and aspiration pneumonia was strongly suspected. Due to a grave prognosis he was humanely euthanized.

**Gross Pathology:** The subcutaneous tissues, mucous membranes and fat were diffusely discolored yellow. The femoral bone marrow cavities were filled with dense bone matrix and small amounts of bone marrow, which sank in water.

Approximately 200 ml of yellow-red serosanguineous fluid with fibrin tags was present within the abdominal cavity. The liver weighed 1070 g (6.7% of body weight), was moderately enlarged and firm, with a dark red-brown granular capsular surface and multifocal 1- 2 mm in diameter circular firm tan foci within the parenchyma. The hepatic lymph nodes were prominent with normal architecture. The spleen was dark pink and enlarged, measuring 26 x 9 x 2.5 cm. On the capsular surface, there were multifocal 2-3 mm blue to green plaques (siderofibrotic plaques).

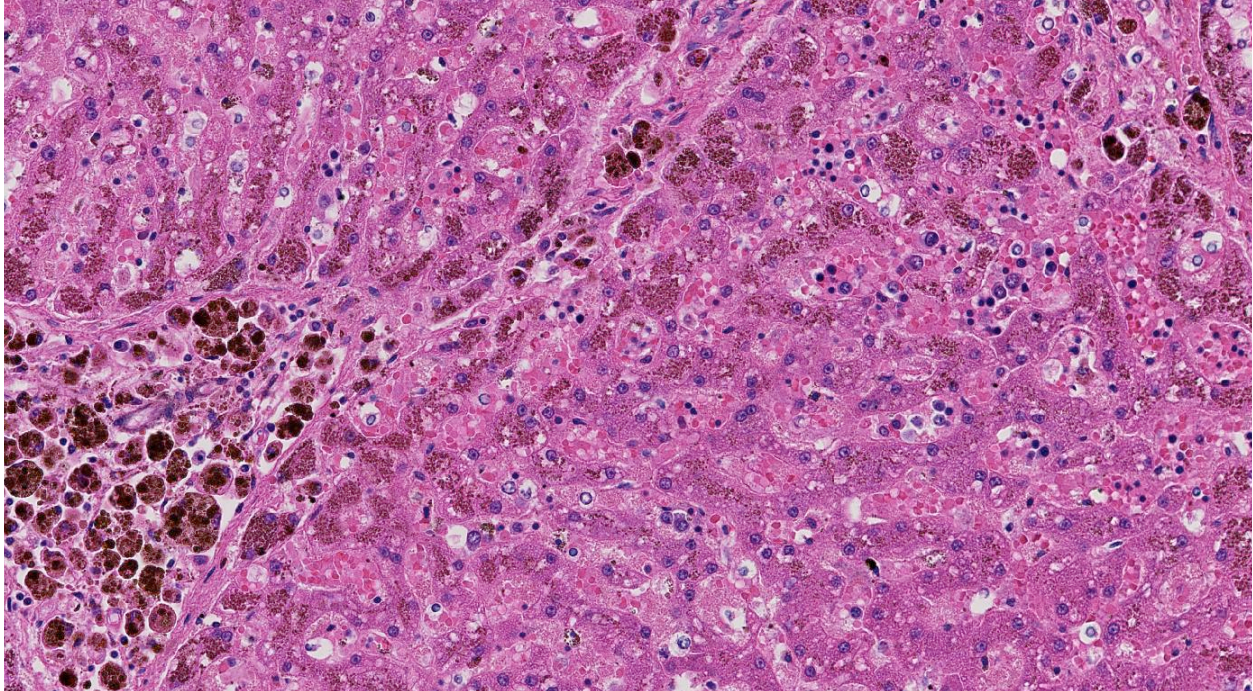
There was 100 ml of red serosanguineous fluid in the thoracic cavity. The sternal lymph nodes were prominent with normal architecture. There was 20 ml of red serosanguineous fluid within the pericardium, and the heart weighed 180 g (1.1% of body weight). The ventral portions of the right cranial, middle and caudal lung lobes were red brown and firm. The left cranial lung lobe contained a 4-5 cm long depressed firm tan streak on the serosal surface. The right middle lung lobe sank in formalin.

**Laboratory Results:** Serial CBCs: PCV/Hct persistently ranging from 15-20%, with reticulocyte counts >170 K/uL.

- Direct antiglobulin (Coombs') test: Negative
- Erythrocytic osmotic fragility testing: Normal
- **Postmortem liver iron (ICP/MS): 37,300 ppm dry weight** (reference range canine: 350-1200 ppm dry weight, 100-300 ppm wet weight). Most published reference ranges are reported on a wet weight basis, dry weight results are expected to be 3.5 – 4 X higher than wet weight.
- DNA-based screening test for known PK mutations in Basenjis, Beagles and West Highland White terriers (8): Negative
- **DNA sequencing and mutation results: Homozygous single base missense mutation of PK-LR gene (8)**
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**Histopathologic Description:** Liver: Multifocally within the hepatic parenchyma, portal areas are moderately to markedly expanded by increased numbers of macrophages that contain abundant dense dark brown to black globular pigment (hemosiderin), increased bile duct profiles, and bands of loose fibrous connective tissue that occasionally bridge adjacent portal areas, and less frequently, centrilobular regions. Fibrous connective tissue multifocally surrounds and individualizes periportal hepatocytes, which often contain intracytoplasmic finely granular dark brown to black pigment. Hepatocytes in the surrounding lobules also contain variable amount of a similar cytoplasmic pigment. Hepatic lobules are irregular in size and there are multifocal nodules of regeneration. Frequently within sinusoids and portals areas there are scattered aggregates of





3-1. Liver, dog: Hepatocytes, most prominent in periportal areas accumulate brown pigment, which is also present within large amounts of macrophages in portal areas (left). (HE 188X)

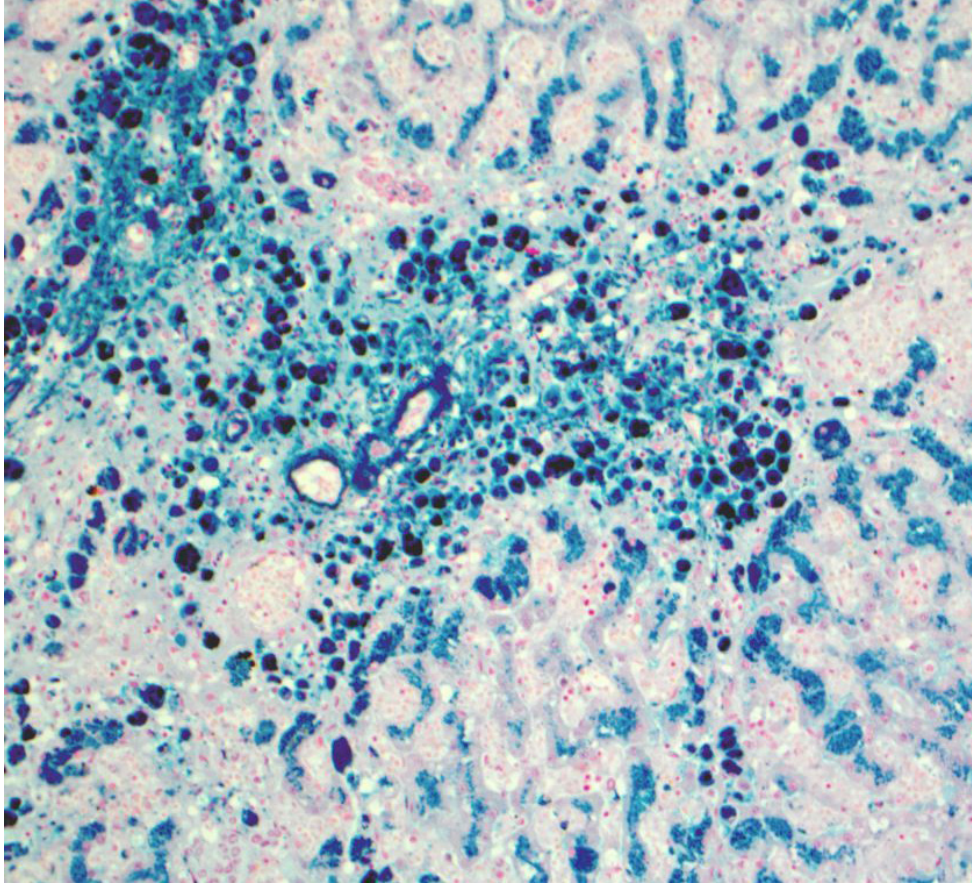
primarily erythroid and fewer myeloid precursors (extramedullary hematopoiesis), as well as rare individual hepatocytes which are shrunken with hypereosinophilic cytoplasm and pyknotic nuclei (necrotic). Occasionally within connective tissue in portal areas and within blood vessel walls there is granular to globular basophilic material (mineral). Multifocally on the capsular surface and extending into the underlying hepatic parenchyma, there are regionally extensive areas of fibrosis with increased bile duct profiles (post-necrotic scarring).

Liver (Perl's iron, Figures 1A and 1B): Globular pigment within hepatocyte, macrophage and Kupffer cell cytoplasm in portal areas and extending into adjacent hepatic lobules stains positively, confirming the presence of iron.

Lymph node (hepatic, Figure 2): Multifocally, medullary cords are moderately expanded by large numbers of macrophages that contain abundant dense dark brown to black globular pigment (hemosiderin), intracytoplasmic fragments of erythrocytes (erythrophagocytosis) and scattered foci of extramedullary hematopoiesis. In these regions, connective tissue and blood vessel walls often contains mineral. A mild drainage reaction is present.

**Contributor's Morphologic Diagnosis:** Liver: Moderate multifocal chronic periportal and hepatocellular hemosiderosis, portal-portal and portal-central bridging fibrosis with nodular regeneration and bile duct hyperplasia (secondary hemochromatosis, cirrhosis); extramedullary hematopoiesis, connective tissue mineralization and post-necrotic scarring.

**Contributor's Comment:** Iron overload disorders are defined as either increased deposition of iron in parenchymal organs without clinical manifestations (hemosiderosis) or by organ dysfunction secondary to iron-induced injury (hemochromatosis).<sup>11,16</sup> Hemochromatosis was first described in humans in the 19<sup>th</sup> century based on the classical clinical presentation of bronze pigmentation of the skin, cirrhosis and diabetes mellitus.<sup>14</sup> It is common in humans and some species of birds, but rare in domestic animals.<sup>17</sup> Primary or hereditary hemochromatosis is due to autosomal recessive disorders in iron metabolism. In humans, most are associated with HFE gene mutations. The most common is caused by a single mutation in *HFE* gene; however, other gene mutations have also been described.<sup>1,2,14</sup> A hereditary form of hemochromatosis has also been described in Salers cattle and is similar to what is seen in humans.<sup>10</sup> Hereditary



3-2. Liver, dog: A Perl's iron stain shows accumulation of dark blue granules of hemosiderin within siderophages and blue tint of ferretin within hepatocytes. (HE 200X) (Photo courtesy of: University of Pennsylvania, School of Veterinary Medicine, Laboratory of Pathology and Toxicology and Section of Medical Genetics)

hemochromatosis is more common in humans than secondary; however, causes of secondary include hemolytic anemias +/- chronic transfusions (thalassemia major, sickle cell anemia, and others), dietary iron overload, chronic liver diseases (hepatitis C and B, alcohol-induced liver disease, porphyria cutanea tarda, and fatty liver disease), and other miscellaneous causes.<sup>3,18</sup> In domestic animals, secondary hemochromatosis is rarely seen and has been attributed to repeat blood transfusions, excessive dietary iron, parenteral iron supplementation, primary liver disease and hereditary erythroenzymopathies.<sup>6,10,11,14,16,17</sup>

In the dog of this report, the hemochromatosis is most likely due to the chronic severe hemolytic anemia caused by hereditary PK deficiency.<sup>8</sup> Pyruvate kinase deficiency is an autosomal recessive trait described most commonly in Basenjis, Beagles and West Highland White and Cairn terriers. Affected dogs present with decreased exercise tolerance, tachycardia, systolic

heart murmur, pale mucous membranes and splenomegaly.<sup>4,9</sup> In the past the diagnosis was confirmed by measuring erythrocytic enzyme activity. This can be problematic, as erythrocytes of affected dogs lack the normal adult R-PK isoenzyme, but persistently express the M<sub>2</sub>-PK isoenzyme, which is present in many fetal and adult tissues.<sup>9,17</sup> Testing for known PK-LR gene mutations is simpler and now available for Basenjis, Beagles, West Highland White and Cairn terriers, and Pugs.<sup>7,8,15</sup> The Labrador in this report did not

have any of the known mutations but was homozygous for a single base substitution in exon 7 of the PK-LR gene; this mutation renders the protein dysfunctional.<sup>8</sup>

Pyruvate kinase is primarily responsible for catalyzing the last step in anaerobic glycolysis. A lack of this enzyme greatly impairs ATP-generation in affected erythrocytes, resulting in decreased lifespan and an extravascular hemolytic anemia which is strongly regenerative, with a marked peripheral reticulocytosis.<sup>9</sup> In normal healthy Basenjis, the apparent erythrocyte half-life is approximately 10-28 days, but only 5.8 days in affected dogs.<sup>6</sup> It is estimated that this rapid erythrocyte turnover results in a plasma iron turnover rate that is twice normal. Prolonged and progressive iron overload frequently results in hemosiderosis and may lead to bridging portal fibrosis (cirrhosis) and thus hemochromatosis in the liver of affected dogs.<sup>6,11,12</sup>

Tissue is damaged by excessive uptake of iron as non-transferrin bound iron (NTBI), which, if it accumulates at sufficient concentrations, results in free radical production, iron-induced lipid peroxidation and organelle dysfunction, such as mitochondrial death.<sup>13,14</sup> The organs most commonly affected due to excessive cytoplasmic iron deposition include the liver and pancreas, but can also occur in lymphoid organs, as well as myocardial and skeletal muscles.<sup>10,14,16</sup> Fibrosis and cirrhosis develop in people at a threshold level of approximately 22,000 ppm per dry weight matter (6600 wet weight), which is 12 x normal.<sup>10</sup> The postmortem liver iron level in this dog was 31 x the normal upper reference range limit for dogs, resulting in the changes observed within the hepatic parenchyma.

The lesions associated with hemochromatosis occur when there is loss of equilibrium in systemic iron homeostasis, which is a fine balance between absorption and loss. Control of intestinal iron absorption is tightly regulated by multiple genes and proteins, as there is apparently no regulated mechanism for hepatic or renal excretion of iron in mammals, which occurs only through loss of body secretions, desquamation of intestinal and epidermal cells, or bleeding.<sup>13</sup> Absorption occurs in the duodenum and proximal jejunum across the basolateral membrane of enterocytes where it is taken up as two forms: heme iron from the digestive breakdown of hemoglobin and myoglobin, and non-heme iron released from vegetarian food sources. One of two proteins then sequester the iron to keep it non-reactive: ferritin, which stores iron in cells and is the precursor to hemosiderin, or transferrin, which is the principal iron carrying protein in plasma that distributes iron among tissues for use in biosynthesis of hemoglobin and other iron-containing proteins, and transports to hepatocytes for storage or to tissue macrophages that phagocytize senescent erythrocytes and recycle iron.<sup>1,11,13</sup>

Much recent work has shown that the liver plays a central role in sensing iron needs, which is modulated in part by hepcidin, an acute phase protein which is produced in the liver and secreted into the circulation.<sup>1,3</sup> It is thought that hepcidin responds to two independent signals, iron level and inflammatory status.<sup>13</sup> Hepcidin is upregulated when increased plasma iron levels are sensed, and inhibits export of iron by enterocytes

and iron laden macrophages, thus participating in a negative feedback mechanism of regulation.<sup>11,13</sup> It functions by binding to the iron export protein ferroportin, and induces its degradation.<sup>5</sup> Macrophage-based cytokines IL-6, IL-1 and tumor necrosis factor- $\alpha$  also stimulate hepatocellular synthesis of hepcidin.<sup>13</sup> This mechanism is proposed to limit the availability of iron to infectious agents and tumor cells, which require iron to proliferate.<sup>13</sup> Decreased hepcidin expression in dogs with induced iron deficiency has recently been validated, and this work provides incentive for further investigation of gene and protein expression of this and other iron regulating molecules in the dog.<sup>5</sup>

**JPC Diagnosis:** Hepatocytes: Siderosis, periportal, diffuse, severe, with periportal hepatocellular loss and bridging fibrosis.

**Conference Comment:** As elaborately summarized by the contributor, there are serious consequences to a mutation of the rate-limiting step of the anaerobic glycolytic pathway. Erythrocytes, which lack mitochondria, are solely dependent on this pathway for ATP production and thus are most severely affected. However, with the compensatory extramedullary hematopoiesis that can occur following the rapid turnover of erythrocytes, it is ultimately the accumulation of iron within the liver which causes the clinical deterioration observed in this case.

Osteosclerosis with the loss of marrow observed grossly in this case is consistent with other reports in dogs and does not occur in people or cats with PK deficiencies.<sup>8</sup> Mutations resulting in a deficiency of the enzyme phosphofructokinase, another protein in the glycolytic pathway, has also been described in dogs. It causes a persistent hemolytic anemia with occasional episodes of intravascular hemolysis due to alkalemia from hyperventilation, such as occurs during strenuous exercise. This also leads to hepatic hemosiderosis and can affect the skeletal muscle, however, osteosclerosis and liver failure has not been observed in these cases.<sup>9</sup>

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**CASE IV:** 10727798 (JPC 3166453).

**Signalment:** 10-year-old spayed female mixed breed dog (*Canis familiaris*).

**History:** Patient was initially being evaluated for a left mandibular mass. On additional imaging studies, other tumors were found in the left cranial lung lobe and left adrenal gland. The patient then underwent thoracotomy for partial lung lobectomy and the specimen was submitted for histopathological examination. Two weeks later, the dog underwent laparotomy for excision of the left adrenal gland, and an additional mass was noted in the right medial hepatic lobe. The patient never recovered from anesthesia after laparotomy and developed acute hemoabdomen and hematemesis. Samples from the hepatic mass and the left adrenal gland were submitted for histopathologic examination.

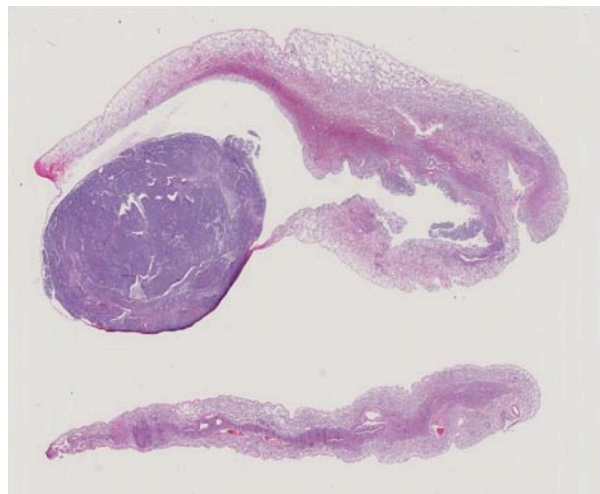
**Gross Pathologic Findings:** The sample from the left cranial lung lobe contained a 7 mm diameter, well demarcated subpleural nodule at the distal border of the lobe (Fig.1). On cut section, this nodule was firm and pale tan. Approximately 3mm cranial to this nodule there was an irregular and poorly demarcated area of parenchymal cavitation immediately adjacent to the inked (yellow) surgical margin.

**Histopathologic Description:** A section of lung contains an unencapsulated and well-demarcated peripheral subpleural mass. This mass is solid and composed of two distinct cellular populations,

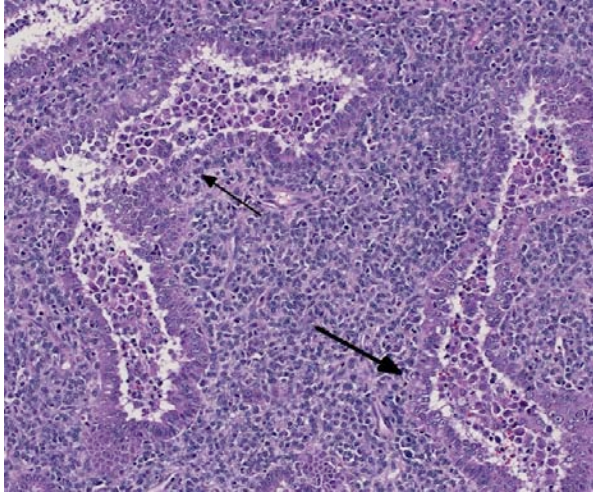
characterized by large scattered acinar-like structures and peripheral papilliferous projections (adenocarcinomatous component), amongst tightly packed cords of cells (small cell component), which diffusely obscure a background of fine fibrovascular stroma. The acinar-like structures are lined by a monolayer of tall columnar epithelium with abundant well-delineated acidophilic cytoplasm. The nuclei are round to ovoid and have sparse finely stippled chromatin with small conspicuous nucleoli. There is loss of nuclear polarity. Anisocytosis and anisokaryosis are moderate, up to 2 fold, with 0-1 mitotic figures per high power field (40X). The cells between the acinar structures are smaller, round to polygonal and have moderate to poorly delimited light acidophilic to clear cytoplasm. The nuclei are round to ovoid and have sparse finely stippled chromatin with large conspicuous nucleoli. Anisocytosis and anisokaryosis are moderate to marked, up to 3 fold, with 8-10 mitotic figures per high power field (40X). There is extensive coalescing necrosis of the mass. Abundant neutrophils, macrophages, sloughed neoplastic cells and basophilic mucus fill the lumen of the neoplastic acini. Small numbers of neutrophils, fewer eosinophils, lymphocytes and rare plasma cells infiltrate the mass. The inked surgical margin is clean but narrow and measures less than 1 mm thick. The pulmonary parenchyma adjacent to the mass is moderately atelectatic and displays extensive hemorrhage within alveolar spaces.



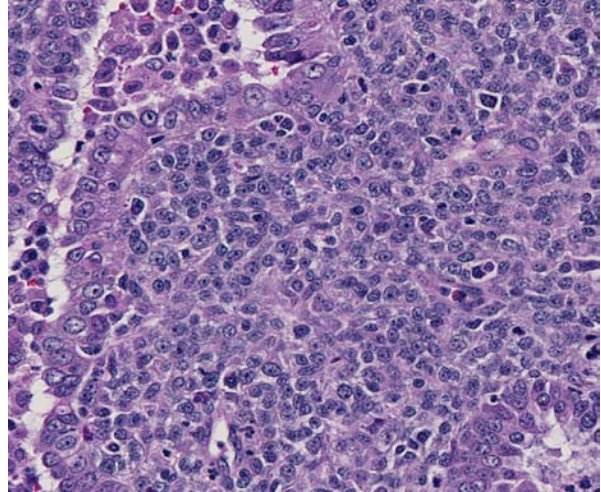
4-1. Lung, dog: The left cranial lung lobe contained a 7 mm diameter, well demarcated subpleural nodule at the distal border of the lobe. (Photo courtesy of: The Animal Medical Center, 510 East 62<sup>nd</sup> St. New York, NY- www.amcny.org)



4-2. Lung, dog: Subgross of the neoplastic nodule. (HE 6.3X)



4-3. Lung, dog: The neoplasm is composed of acini lined by neoplastic columnar epithelial cells (arrows), which are separated by neoplastic polygonal epithelial cells arranged on a moderate fibrous stroma. (HE 100X)



4-4. Lung, dog: Higher magnification of neoplastic cells, with acini recapitulating primitive airways. (HE 320X)

With immunohistochemistry, the adenocarcinomatous component of the tumor was strongly positive for pancytokeratin markers AE1 and AE3. Although pancytokeratin expression was negative for the vast majority of the small cell component, scattered individual cells displayed strong cytoplasmic expression for both markers. Neither the adenocarcinomatous or small cell component expressed synaptophysin, chromogranin or neuron specific enolase (NSE). Small numbers of scattered cells throughout the tumors expressed CD20 and CD18 and were interpreted in combination with the H&E section as leukocytes (secondary inflammation).

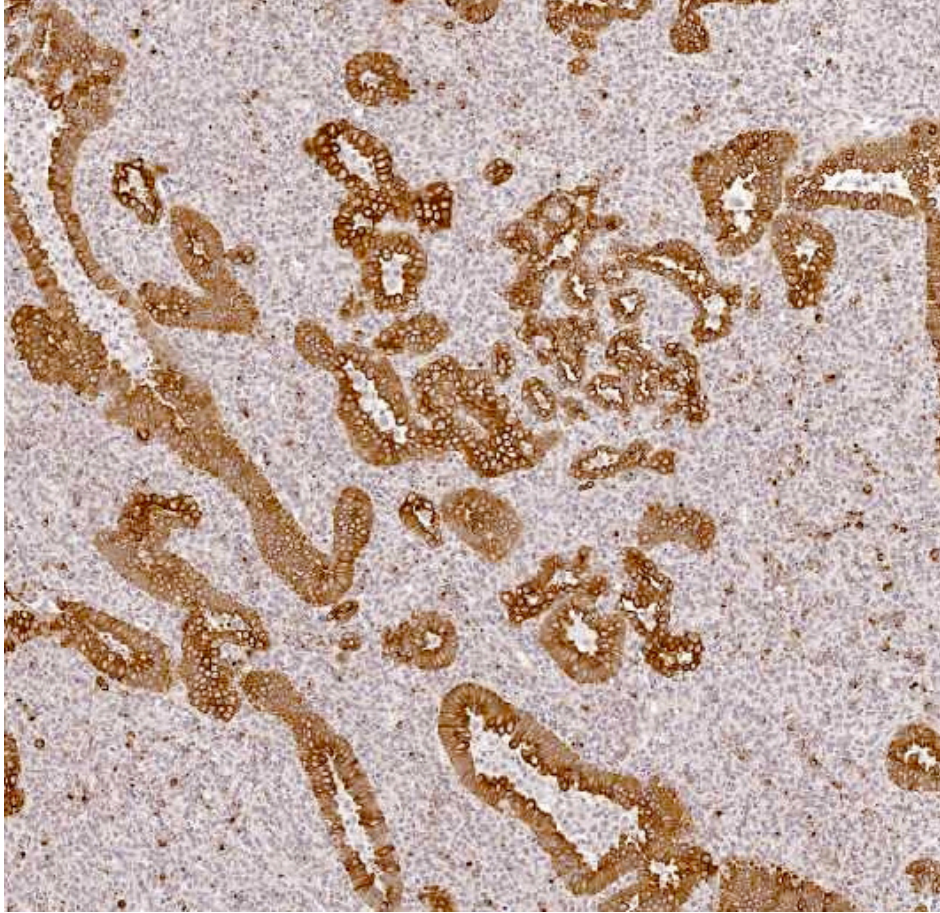
Histopathologic examination of the left mandibular mass (not provided) was consistent with a salivary gland adenocarcinoma. Sections from the left adrenal gland (not provided) revealed a malignant pheochromocytoma. The hepatic nodule from the right medial lobe (not provided) displayed the same morphology as observed in the pulmonary nodule and was interpreted as a focus of metastasis from the pulmonary neoplasm.

**Contributor's Morphologic Diagnosis:** Lung (left cranial lobe): Combined pulmonary carcinoma.

**Contributor's Comment:** The dog in the present case had three distinct unrelated neoplasms in different organs with no overt clinical evidence of metastatic disease, except for a hepatic nodule, which was later interpreted as a focus of

metastasis from the primary pulmonary mass. The cause for initial presentation to our hospital was a left parotid salivary adenocarcinoma, and when a pulmonary nodule was found in subsequent imaging studies, the immediate clinical suspicion was of metastatic disease from the parotid gland to the lungs. Surprisingly, the pulmonary nodule exhibited unique dual microscopic morphology (an adenocarcinomatous and a small-cell component), not observed in the salivary gland mass, and was diagnosed as a combined pulmonary carcinoma.

Combined tumors are neoplasms that display more than one cellular morphology and microscopic architecture, often composed of cells with remarkably different histological, histochemical and immunohistochemical properties.<sup>8</sup> One example of combined tumor in humans is combined pulmonary carcinomas, which have been reported to exhibit combinations of up to 4 distinct morphologies (squamous cell carcinoma, small-cell/neuroendocrine carcinoma, blastoma and adenocarcinoma).<sup>9</sup> Additionally, extra-pulmonary combined tumors with a small cell/ neuroendocrine component associated with a component of squamous cell carcinoma or adenocarcinoma appear to be relatively common in the rare group of small cell carcinomas in people, and have been reported in the esophagus, prostate gland and colon.<sup>10</sup> Aside from combined pulmonary carcinoma of dogs and cats, other examples of combined tumors in domestic animals include carcinosarcomas of mammary



4-5. Lung, dog: Neoplastic cells lining airways stain strongly positive for cytokeratin; polygonal cells multifocally stain positively as well. (Anti-cytokeratin 100X)

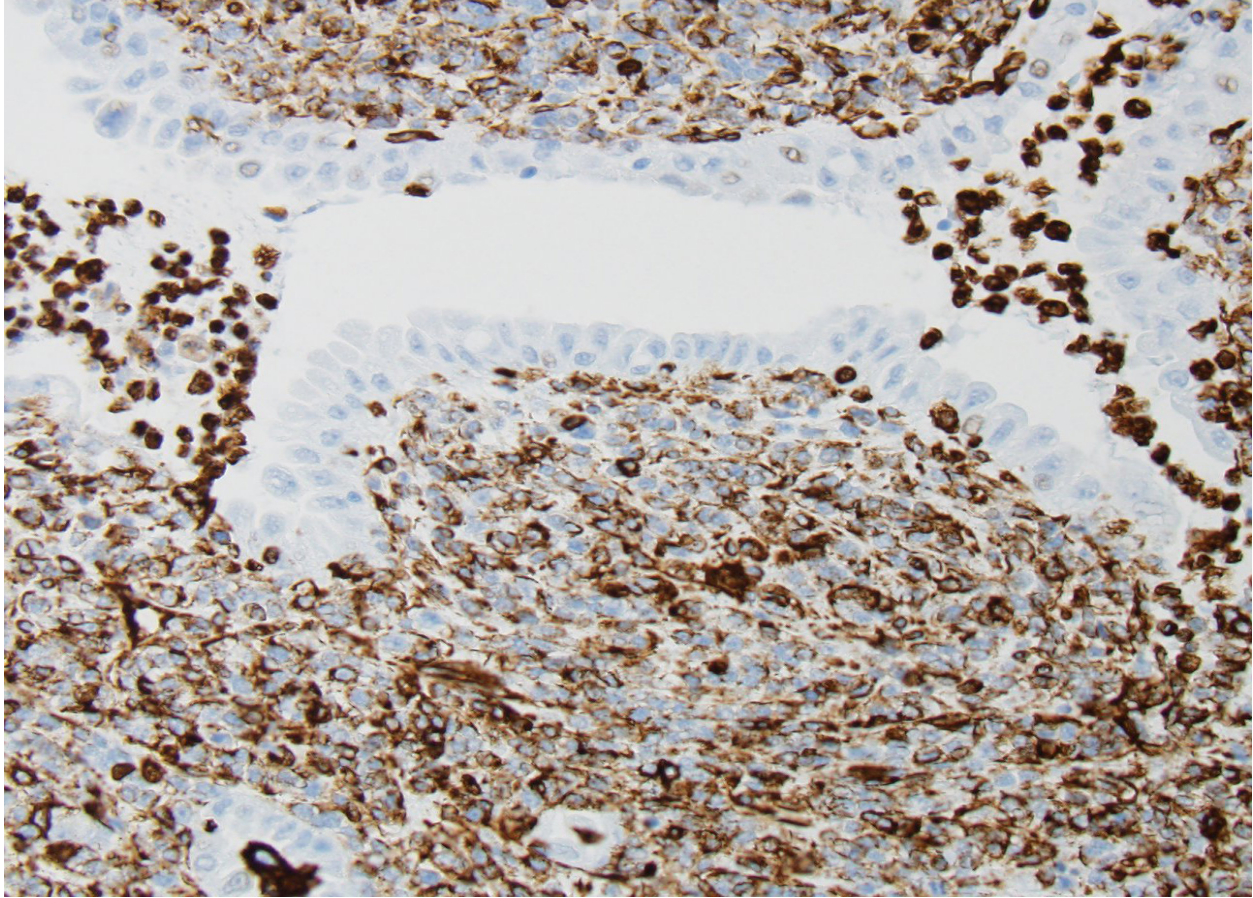
gland and combined hepatocellular-cholangiocellular carcinoma.<sup>13</sup>

Overall, primary pulmonary neoplasms are exceedingly common in people and uncommon in dogs.<sup>1</sup> The prevalence of primary lung cancer in the overall canine population has been reported to be of 1% of all diagnosed canine neoplasia.<sup>1,3,12</sup> The most accepted veterinary classification of pulmonary neoplasms to date includes adenomas and papillomas, bronchial gland carcinoma, squamous cell carcinoma, adenocarcinomas of papillary, acinar, solid and mixed types, bronchioloalveolar carcinoma, adenosquamous carcinoma, small and large cell carcinomas, neuroendocrine carcinoma, pulmonary blastoma and combined carcinoma.<sup>4,14</sup> Adenocarcinomas appear to be the most common type of lung cancer in domestic animals.<sup>3</sup> In contrast to the veterinary classification, in people, combined carcinoma is classified as a subvariant of small cell carcinomas, since it has been shown that the

small cell component in those tumors has neuroendocrine properties. The importance of proper tumor classification in this instance lies in the fact that prognostic outcome and therapeutic management largely depend on the leading histomorphological tumor type.<sup>6</sup> The same has not been proven in the rare cases of combined pulmonary tumors of domestic animals<sup>4</sup>, where the small cell component has failed to express the most common neuroendocrine immunohistochemical markers, such as chromogranin, synaptophysin and neuron-specific enolase (as in the present case), and accurate definition of histogenesis of this

component remains unavailable. To date, the histogenesis of the small cell component of combined pulmonary tumors of domestic animals has only been morphologically and immunohistochemically characterized as a pulmonary basaloid cell. The difficulty in further analyzing these tumors at the molecular level in domestic animals is most likely due to the rarity of the tumor, which has only been reported in cats and less commonly dogs.<sup>4</sup>

Despite the high prevalence of lung cancer in people, combined pulmonary tumors are also rare in this population. In a recent retrospective study of 1,158 pulmonary surgical specimens of people, combined carcinomas represented 1.8% of all pulmonary tumors.<sup>5</sup> Nevertheless, a few studies in human specimens have been able to provide further insight on what seems to be the most intriguing and fascinating aspect of this rare neoplastic entity; the nature of its capability to produce such phenotypically distinct components.



4-6. Lung, dog: Neoplastic polygonal cells stain strongly positive for vimentin. (Anti-vimentin 280X)

The main questions raised around this entity are whether the different components of the tumors are genetically related and why and how differentiation towards such different cellular components occurs.<sup>5,8,9,11</sup> Answer to these questions may shed light on the histogenesis and biologic behavior of the tumor cells. Clonality assays and study of LOH (loss of heterozygosity) have been employed to aid in answering these questions; however, so far, the results raise more questions than definitive results.

To date, a few studies have shown monoclonality and identical genetic alterations in the specific chromosomes analyzed in a few of these tumors, raising the hypothesis that the different cellular components of the tumors are likely derived from a common precursor cell and driven by the same carcinogens.<sup>5,8,9,11</sup> In these cases, it remains to be understood why cells with a common genetic profile display such distinct morphology, histochemical and immunohistochemical properties. It can be hypothesized that during malignant transformation, some of the progeny

cells derived from a common precursor cell may convert to a different morphology under influence of different environmental stimuli. Alternatively, the clonal neoplastic cell may retain the ability to differentiate toward other morphologic phenotypes in response to different stimuli.<sup>8</sup>

In contrary to those results, one study has shown a few combined tumors with different genetic profiles, despite such close morphologic relationship of the different cellular components. In those cases, it was hypothesized that the different groups of cells (small cell component with squamous cell differentiation and a separate adenocarcinomatous component) may have undergone separate progression from a pluripotent single clone in a very early stage, resulting in distinct genetic abnormalities that may have developed in a later phase of the tumor progression.<sup>11</sup> Further clonality and LOH studies on this rare neoplastic entity are still necessary and would help to provide additional insight in the field of stem cells, tumor biology and oncologic pathology.



The concomitant development of multiple different neoplasms in this dog (salivary gland adenocarcinoma, combined pulmonary carcinoma and pheochromocytoma) is also an interesting point of discussion beyond the scope of this brief review.

**JPC Diagnosis:** Lung: Combined pulmonary carcinoma.

**Conference Comment:** This case provides an exceptional example of a rarely reported tumor in dogs and cats. As the contributor elucidates, the histogenesis of this neoplasm is not definitive which offers an interesting opportunity for discussion and speculation surrounding this case. The additional clinical findings of two other distinct neoplasms and liver metastasis in this dog further adds to the discussion with regard to tumor suppressors and malignant transformation.

As previously outlined, the reactivity of cytokeratin within both cell populations is characteristic to this diagnosis. The conference discussion, however, was focused on the finding of diffuse immunoreactivity among the small cell population with vimentin leading some to consider the diagnosis of carcinosarcoma for this case. Carcinosarcomas usually have areas of osseous or chondrous metaplasia within the mesenchymal cell population<sup>4</sup>; and the small cell component in this case does not have spindle cell morphology, thus our agreement with the contributor's diagnosis.

Participants noted the primitive morphology and loss of polarity within the small cell component. This led to a discussion on the epithelial-mesenchymal transition (EMT) as being a possible cause for the change in cellular morphology as well as the expression of vimentin, which would be consistent with the presence of metastasis observed in this case.

The EMT is a well-defined process of transformation during which neoplastic cells acquire the ability to invade and disseminate. Included in this transformation is a conversion from a polygonal to spindle morphology along with the repression of E-cadherin expression.<sup>7</sup> Loss of E-cadherin, a key cellular adhesion molecule, eliminates the cellular restraint mechanism within the tissue of origin thereby permitting dissemination. This transformation has

been characterized histologically by increased expression of vimentin, which also has been found to correlate with grading criteria in some neoplasms.<sup>15</sup> While there have been studies examining cytokeratin and vimentin expression in canine pulmonary tumors, a correlation with histologic grade has not been demonstrated.<sup>2</sup>

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