



Conference 4 1 October 2008

Conference Coordinator:
Todd M. Bell, DVM,

Wednesday Slide Conference

Moderator:

Taylor Chance, DVM, Diplomate ACVP

CASE I – 08-0013-03 (AFIP 3102187)

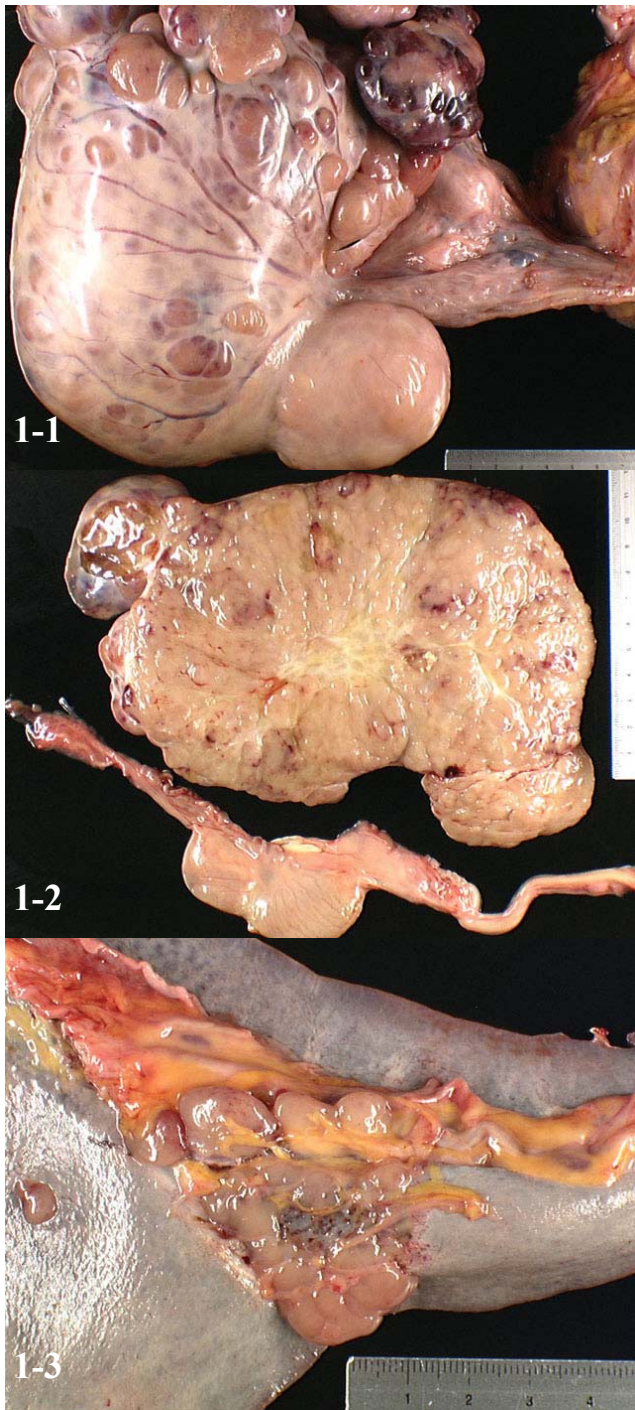
Signalment: 18-year-old, male, pony, equine, *Equus caballus*.

History: This pony had a history of progressive apathy and loss of appetite. Medical treatments provided by its veterinarian failed to improve the condition. Two months after the beginning of treatment, the pony was referred to the National Veterinary School of Alfort for severe wasting, rigidity of the gait, edema of the hindlimbs and a cardiac murmur. Echocardiogram and Doppler measurements revealed a moderate aortic stenosis without apparent cardiac repercussion. The ultrasonic examination of the abdominal cavity revealed parenchymal heterogeneity of the left kidney and several voluminous abdominal masses. The patient was euthanized due to poor prognosis, poor condition, and for economical reasons. It was then submitted to necropsy.

Gross Pathology: A general severe amyotrophy and a mild dehydration were noted at necropsy. Approximately 200 mL of a clear red-tinged fluid was present in the peritoneal cavity (ascites). Multiple nodules of mineralization were observed in the hepatic parenchyma accompanied by a multifocal villous perihepatitis, probably secondary to migration of strongyle larvae.

A bilateral cryptorchidism was detected with both testicles being in an intra-abdominal position. The left testicle was severely enlarged (10 x 12 x 20 cm), lobulated, white and moderately firm with foci of haemorrhage and mineralization (**Fig. 1-1, 1-2**). Another mass of similar size and consistency was detected in the left iliac region (most likely left iliac lymph node) around the left ureter and left iliac artery, and vein. Multiple white and moderately firm nodules were attached to the splenic hilus. We made a tentative gross morphologic diagnosis of seminoma with nodal and splenic (most likely by transcoelomic implantation) metastases (**Fig. 1-3**).

Multifocal endocardial mineralizations, appearing as plaques were observed in the ventricles and atria, particularly in the left side, and in the aortic intima. Mineralization was severe on the sigmoid aortic valves. Multifocal myocardial necrosis and mineralizations were suspected as well. The tracheal mucosa contained small mineralized granules, giving it an appearance of abrasive paper. Mineralizations were also observed in



1-1. Testicle, horse. Seminoma of the left testis.

1-2. Testicle, horse. Seminoma in left testicle (top) with hypoplastic right testicle (bottom). Note the overall conserved shape of the left testicle with its epididymis despite the massive tumoral infiltration. Foci of necrosis, haemorrhage and mineralization are present in the left testis.

1-3. Spleen, horse. Metastasis of seminoma in the splenic hilus.

Gross photographs courtesy of National Veterinary School of Alfort (France) ECOLE NATIONALE VETERINAIRE D'ALFORT Unité d'Histologie et d'Anatomie Pathologique 7, avenue du Général de Gaulle 94704 Maisons-Alfort Cedex France.

the renal cortex of both kidneys. As parathyroid glands were macroscopically normal, we suspected these lesions to be metastatic calcifications due to humoral hypercalcemia of malignancy (see Contributor's Comment).

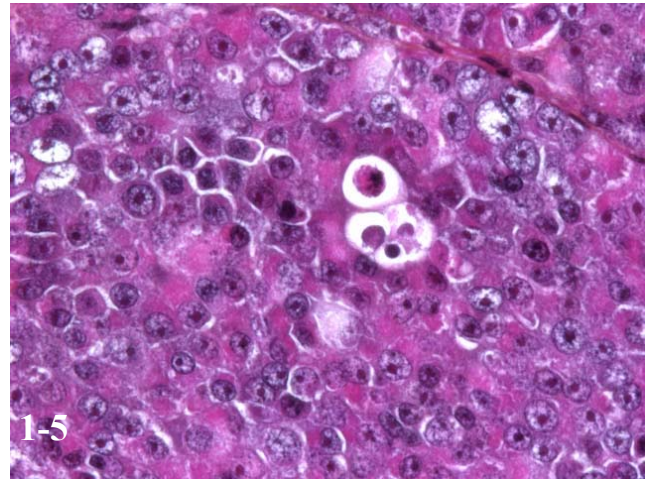
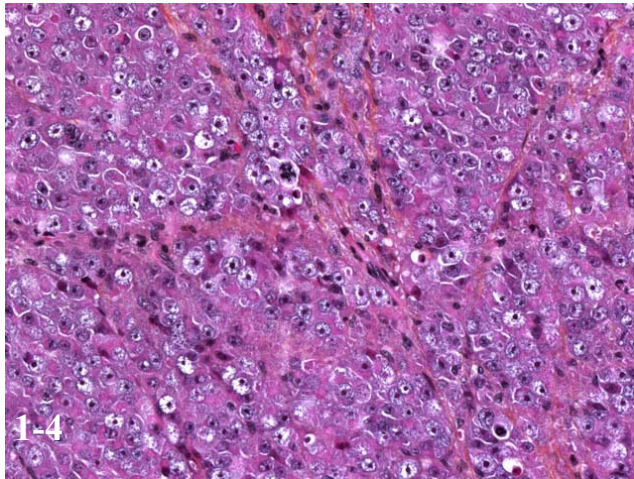
The right testicle was moderately reduced in size (6 x 3 x 3 cm) with a spongy consistency (**Fig. 1-2**). We made a tentative gross diagnosis of testicular hypoplasia.

Subcutaneous tissue in caudoventral abdomen and hindlimbs was severely edematous. Because of tumoral involvement of the left iliac nodes and vessels, we hypothesized the acquired lymphedema was secondary to obstruction of lymph flow by the neoplasm.

Histopathologic Description: Left testicle: An encapsulated and well demarcated tumoral proliferation with high cellular density infiltrates and effaces the testicular parenchyma sparing some epididymal tubules (not present in all slides). Multifocally, infiltration of the testicular capsule is observed. Neoplastic cells are arranged in densely packed sheets, nests, and lobules on a fine fibrovascular stroma. Cells are non-cohesive, round, and 20 to 25 µm in diameter with indistinct cell borders. Nuclei are centrally located, large, oval, vesiculate, and contain one prominent eosinophilic nucleolus and marginalized finely reticulated chromatin. The cytoplasm is abundant, eosinophilic, and finely granular. There is marked anisocytosis/anisokaryosis and a moderately high mitotic index (2-3 mitoses per HPF). There is multifocal individual cell necrosis ("starry-sky pattern") (**Fig. 1-4, 1-5**). Only a few lymphocytes are present between the tumoral cells. Some foci of mineralization are also observed.

Spleen: The peritoneal side of the splenic capsule is infiltrated by a similar tumoral proliferation (not obvious in all slides). Intimal bodies and diffuse intimal mineralizations are prominent in some arteries.

Right testicle: Testicular tubules are rarefied and separated by large bundles of a dense, mature fibrous



1-4. Testicle, horse. Seminoma. Normal testicular architecture is effaced by round to polygonal cells with variably distinct cell borders, moderate amounts of eosinophilic granular cytoplasm and generally round nuclei with prominent nucleoli. Mitotic figures range from 3-5 per HPF.

1-5. Testicle, horse. Seminoma. Higher magnification demonstrating single cell necrosis.

Photomicrographs courtesy of National Veterinary School of Alfort (France) ECOLE NATIONALE VETERINAIRE D'ALFORT Unité d'Histologie et d'Anatomie Pathologique 7, avenue du Général de Gaulle 94704 Maisons-Alfort Cedex France.

connective tissue. They have a markedly diminished number of germinal cells and no spermatozoa with normal to mildly decreased numbers of Sertoli cells, interstitial cells and efferent ductules. Basal membranes of tubules are normal in thickness and are not obviously wrinkled. Some interstitial cells contain brown granular pigments (probably lipofuscin granules).

Contributor's Morphologic Diagnosis:

1. Left testicle: seminoma, diffuse-type
2. Splenic capsule: metastasis of seminoma
3. Right testicle: testicular hypoplasia

Contributor's Comment: This case highlights the relationship between cryptorchidism and testicular tumor development and hypoplasia.

Primary testicular tumors may be classified as *sex-cord stromal (gonadal stromal) tumors*, which include Leydig (or interstitial) cell tumor and Sertoli cell tumor, and *germ cell tumors*, which include seminoma, embryonal carcinoma and teratoma. *Mixed germ cell-sex cord stromal tumors* are rare and include gonablastoma. Tumors derived from other testicular elements such as mesothelioma, hemangioma, fibroma and their malignant counterparts are infrequent.

Seminomas are common in canine and equine testis.

They occur less frequently in the ram, buck and bull. This type of tumor is mostly seen in older animals and is very common in cryptorchid testicles.(2,6) Seminoma is the second most common canine testicular neoplasm and the most common testicular neoplasm in the aged stallion.(2,3,6) Clinically, this type of tumor can be recognized in horses by ultrasonic examination. A diffuse heterogeneous appearance with diffuse hypoechogenicity of the testis and ill-defined nodular regions of hyperechogenicity are characteristic of equine seminomas.(1)

Seminomas develop presumably from basal spermatogonia of the seminiferous tubules. These tumors are seldom malignant and show no hormone production. However, they tend to be locally invasive and there is no known factor to predict their metastatic potential. The actual WHO Classification of Domestic Animals does not recognize a traditional distinction between benign and malignant forms of seminomas, the term "seminoma" being applied for both. This may reflect in part the influence of human pathology where seminomas are considered malignant (see below). Because of their tendency to be locally invasive with rare metastasis in domestic animals, we should regard them as tumors of generally low malignancy. However, they are more likely to have malignant behaviour than Sertoli and Leydig cell tumors, particularly in dogs and horses.(2,6)

Table 1-1: Human WHO classification of germ cell tumors of the testicle (9)

(0) = benign; (1) = borderline or uncertain behaviour;

(2) = Carcinoma *in situ* or grade III intraepithelial neoplasia; (3) = malignant

Intratubular germ cell neoplasia, unclassified (IGCNU) (2) Other types

Tumors of one histological type (pure forms)

Seminoma (3)

Cribiform, pseudoglandular and tubular variant

Seminoma with syncytiotrophoblastic cells

Spermatocytic seminoma (3)

Spermatocytic seminoma with sarcoma

Embryonal carcinoma (3)

Yolk sac tumor (3)

Trophoblastic tumor

Choriocarcinoma (3)

Trophoblastic neoplasms other than choriocarcinoma

Monophasic choriocarcinoma

Placental site trophoblastic tumor (1)

Teratoma (3)

Dermoid cyst (0)

Monodermal teratoma

Teratoma with somatic type malignancies (3)

Tumors of more than one histological type (mixed forms)

Mixed embryonal carcinoma and teratoma (3)

Mixed teratoma and seminoma (3)

Choriocarcinoma and teratoma/embryonal carcinoma (3)

Others

Seminomas are classified on their histological appearance into the intratubular type or the diffuse type. The earliest development of the tumor is intratubular. Rupture of tubules occurs and the growth becomes confluent, forming broad sheets of closely packed cells. Although not prominent in this case, focal or diffuse accumulation of CD8-positive lymphocytes occurs in most seminomas and is a useful diagnostic feature.(2,4,6) Germ cells express vimentin in a perinuclear pattern, but no expression of neuron-specific enolase nor cytokeratin can be detected by immunohistochemistry. Tumoral cells examined by electron microscopy resemble normal germinal epithelium. A relative scarcity of cytoplasmic organelles, oval nuclei, straight cell borders and a distinct Golgi apparatus are characteristic features of seminomas. (2)

Differential diagnosis on histological examination includes other round cell tumors, particularly malignant lymphoma.

Macroscopically, involved testicles are enlarged, soft to moderately firm (not as firm as Sertoli cell tumors). On cut surface, the tumor has a homogeneous glistening gray/white appearance, resembling lymphoid neoplasms. (2,6)

In humans, the actual WHO classification of germ cell tumors is more complex (Table 1-1).

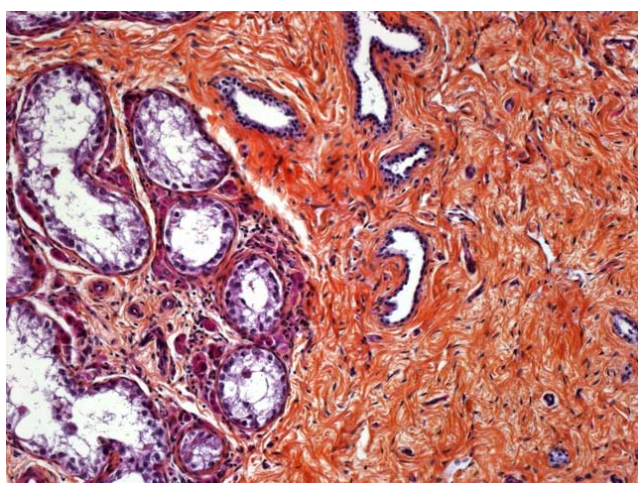
Intratubular germ cell neoplasia, unclassified type (IGCNU) is equivalent to carcinoma *in situ* or intratubular preinvasive tumor but is different from an intratubular seminoma. It is regarded as a precursor lesion and is associated with cryptorchidism or others conditions. Although potentially present in all species, this entity has not yet been described in domestic animals except, interestingly, in horses.(8)

Cryptorchidism is defined as an incomplete descent of the testis and associated structures into the scrotum and is one of most common abnormalities of the male

Table 1-2: Main characteristics of cryptorchidism in domestic animals (2)

Species	Predisposed breeds	Causes	Features
Cat	Mostly Persians	Unknown	Mostly unilateral with no side nor site predilection
Dog	Various	Autosomal recessive suspected in some breeds	Other diseases associated. Right side and inguinal location mainly
	Miniature Schnauzer	Persistent Müllerian duct syndrome (Müllerian duct inhibitory substance insensitivity)	Unilateral or bilateral
Boar	Duroc	Hereditary, recessive (several locus may be involved in Durocs) Mainly due to abnormal development of the gubernaculum	
Bull	Polled Hereford and Shorthorn	Hereditary	Mostly in inguinal region. Left testis twice as often affected than right.
Ram	Polled animals	Autosomal recessive or dominant with incomplete penetrance	Mostly unilateral and involving right testis
Buck	Polled Saanen	Goat polled/intersex syndrome	Right testis mostly
Stallion	No breed predisposition	Unknown	Mainly unilateral. Left testis mostly abdominal and right testis mostly inguinal

reproductive system (the most common in cats and horses). Complete testicular descent usually occurs prior to birth in most species, except in dogs. Retained testes lack spermatogenesis and fertility may be compromised.



Three main stages are defined during the testicular descent: transabdominal migration phase, intra-inguinal phase and extra-inguinal migration. The regulation of descent involves the Müllerian inhibitory substance in the first phase, increased intra-abdominal pressure in the second phase, interaction of androgen, calcitonin gene-related protein, and other factors in the last stage of migration. Thus, multiple mechanisms can be responsible for cryptorchidism.

An association between testicular neoplasia and cryptorchidism is well recognized in several species, especially in dogs. Dogs with abdominally retained testes are most likely to develop Sertoli cell tumor, Seminoma is the second most common type of tumor of abdominally retained testes in the dog. In stallions, retained testicles are prone to develop into seminoma (most likely) or teratoma. Cryptorchidism also predisposes to testicular torsion in dogs and stallions, particularly if there is tumoral involvement. Cryptorchidism also occurs in

1-6. Testicle, horse. Hypoplastic contralateral testicle characterized by few small seminiferous tubules with thickened basement membranes, decreased germinal cells, vacuolated Sertoli cells, and prominent interstitial fibrosis. (Safranin 400X). Photomicrograph courtesy of National Veterinary School of Alfort (France) ECOLE NATIONALE VETERINAIRE D'ALFORT Unité d'Histologie et d'Anatomie Pathologique 7, avenue du Général de Gaulle 94704 Maisons-Alfort Cedex France.

Table 1-3: Main characteristics of testicular hypoplasia in domestic animals (2)

<u>Species</u>	<u>Causes</u>
Cat	Sporadic Cryptorchid and tricolour/calico cats with XXY genotype (Klinefelter's syndrome)
Dog	Hereditary in some breeds. Cause of congenital azoospermia. Associated with cryptorchidism, XX reversal, XX syndrome and treatment of the bitch with diethylstilbestrol.
Bull	Multifactorial, may be hereditary in Swedish Highland breed as a recessive trait with incomplete penetrance: animals with white body and ears are highly susceptible. Most cases of hypoplasia are unilateral with the left side most often affected.
Ram	Sporadic, uni- or bilateral. XXY syndrome reported also. Zinc deficiency implicated in some cases.
Buck	Goat polled/intersex syndrome Sporadic forms of unknown cause in other breeds

boars, bulls and rams.(2,6) **Table 1-2** outlines the main characteristics of cryptorchidism in domestic animals.

Testicular hypoplasia is defined as testes that have failed to grow to normal size and is associated with either cryptorchidism, some intersex conditions, or as an isolated lesion. It can be unilateral or bilateral, and the affected testis can be smaller or of similar size compared to the normal testis. Etiology is often multifactorial and can have a hereditary basis. In humans and mice, a deficiency of gonadotrophins (hypogonadotropic hypogonadism) has been associated with testicular hypoplasia. However, LH and FSH are normal and even elevated in studies concerning domestic animals. Abnormal migration of germ cells to the genital ridge *in utero*, development arrest, or excessive apoptosis play an important role in some forms of the condition. Histologically, there are reduced numbers, length, and/or diameter of tubules. Germ cells may be present or absent, and if present fail to produce spermatozoa. Basement membranes are not particularly wrinkled nor thickened as in testicular atrophy/degeneration.(2) **Table 1-3** outlines the main causes of testicular hypoplasia in domestic animals.

In our case, we had the classical association of cryptorchidism with seminoma and testicular hypoplasia. Furthermore, multifocal severe mineralizations were present in the trachea, aorta, renal tubular and glomerular basement membranes (hypercalcemic nephropathy), and endocardium (**Fig. 1- 7, 1-8, 1-9, 1- 10**) . A moderate hypercalcemia (precise value unknown) was

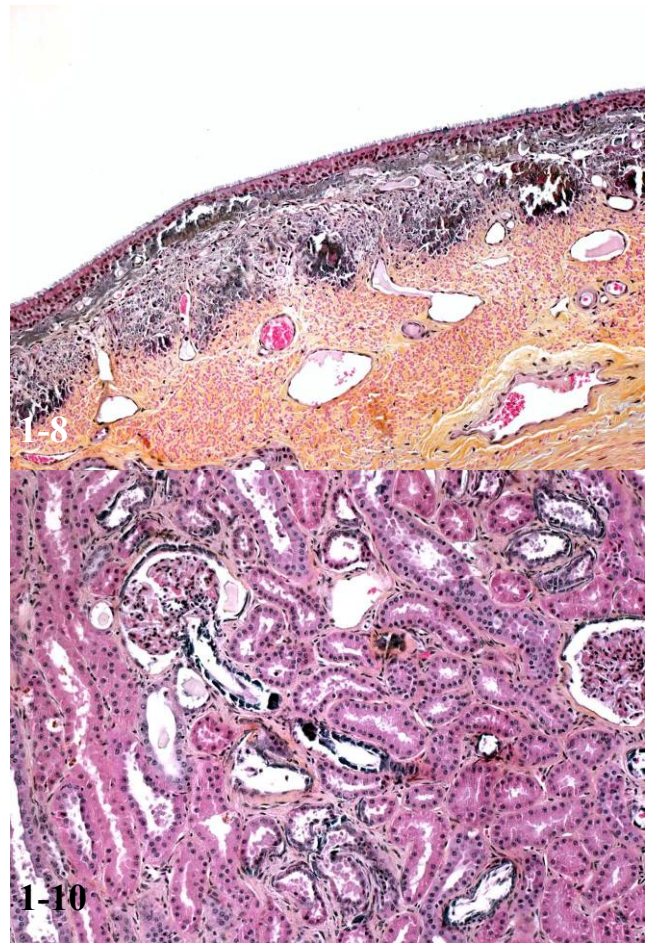
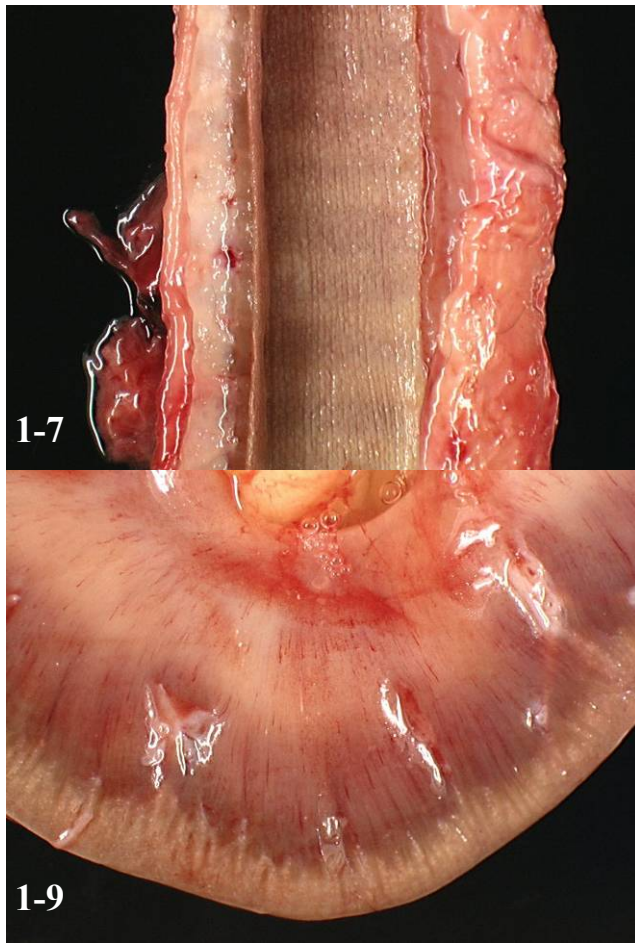
communicated to attending veterinarians by the referring veterinarian. No macroscopic nor microscopic lesions were observed in the parathyroid glands. The ingestion of calcinogenic plants or nutritional imbalances has been excluded by attending clinicians. In such circumstances, the observed mineralizations may reflect a hypercalcemic state, probably induced by the seminoma as a paraneoplastic syndrome (Humoral Hypercalcemia of Malignancy). Interestingly, in human pathology, there are reports of germ cell tumors producing Parathyroid Hormone-related Peptide (PTHrP) with subsequent hypercalcemia.(5,7) To our knowledge, there is no report of paraneoplastic hypercalcemia in connection with germ cell tumors in veterinary medicine. Unfortunately, PTHrP expression could not be investigated in this case.

AFIP Diagnosis:

1. Left testicle: Seminoma, diffuse-type
2. Splenic capsule: Seminoma, metastatic
3. Right testicle: Hypoplasia, diffuse, severe

Conference Comment: A brief review of the gross and histologic appearance of common testicular tumors in dogs was discussed during the conference, and the main points of this discussion are summarized in **Table 1-4**.

The three most common testicular neoplasms in the dog are the Sertoli cell tumor, interstitial cell tumor, and seminoma. Grossly and histologically, Sertoli cell tumors have a prominent fibrous component, while the other two tumors have only a small amount of fibrous



1-7. Trachea, horse. The tracheal mucosa contains small mineralized granules, giving it an appearance of abrasive paper.

1-8. Trachea, horse. Diffuse mineralization in the tracheal mucosa.

1-9. Kidney, horse. Mineralizations in the renal cortex.

1-10. Kidney, horse. Mineralizations in renal tubular and glomerular basement membranes (hypercalcemic nephropathy).

Gross photographs and photomicrographs courtesy of National Veterinary School of Alfort (France) ECOLE NATIONALE VETERINAIRE D'ALFORT Unité d'Histologie et d'Anatomie Pathologique 7, avenue du Général de Gaulle 94704 Maisons-Alfort Cedex France.

stroma. Neoplastic Sertoli cells often palisade along tubules and are the only cells of the testes that are immunohistochemically positive for neuron-specific enolase (NSE). The interstitial cell tumor is derived from interstitial endocrine cells. Grossly, the yellow-orange color of the neoplasm reflects the high level of intracytoplasmic yellow lipochrome pigment. Histologically, neoplastic cells are round to polygonal with granular to vacuolated cytoplasm and multifocal hemorrhagic or cystic areas. Seminomas arise from germ cells and grossly appear as white-gray bulging masses.

Histologically, seminomas are composed of round cells forming either a diffuse or intratubular pattern with a high mitotic rate.(2)

Contributing Institution: National Veterinary School of Alfort (France) www.vet-alfort.fr

References:

1. Beck C, Charles JA, Maclean AA: Ultrasound appearance of an equine testicular seminoma. *Vet Radiol Ultrasound* **42**:355-357, 2001

Table 1-4. Brief review of the gross and histologic appearance of common testicular tumors in dogs (2)

	Gross Appearance	Histologic Appearance
Interstitial cell tumor (Leydig cell tumor)	small, round, well circumscribed minimal distortion of affected testicle bulge on cut section yellow to orange hemorrhagic and cystic	large, uniform round to polygonal cells abundant eosinophilic, granular to vacuolated cytoplasm prominent lipid accumulations rare mitoses
Sertoli cell tumor	well demarcated firm, white irregular, dense bands of connective tissue +/- fluid filled cysts or hemorrhage atrophy of contralateral testicle	tall, slender to columnar cells indistinct cell borders dense, fibrous stroma may palisade along stroma patterns – diffuse and intratubular
Seminoma	lobulated, irregular compresses adjacent parenchyma soft bulging , gray to white to mottled brown	large, uniform, fairly discrete round cells scant cytoplasm occasional giant nuclei multinucleate cells mitoses common

2. Foster RA, Ladds PW: Male genital system. *In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals*, ed. MaxieMG, 5th ed., pp. 572-600. Saunders Elsevier, Edinburgh, London, New York, Oxford, Philadelphia, St. Louis, Sydney, Toronto, 2007

3. Galofaro V, Consiglio C, Rapisarda G, Marino F: Bilateral malignant seminoma with metastases in the mule: a report of two cases. *Reprod Domest Anim* **43**:121-123, 2008

4. Grieco V, Rondena M, Romussi S, Stefanello D, Finazzi M: Immunohistochemical characterization of the leucocytic infiltrate associated with canine seminomas. *J Comp Pathol* **130**:278-284, 2004

5. Looijenga LH, Oosterhuis JW: Pathogenesis of testicular germ cell tumours. *Rev Reprod* **4**:90-100, 1999

6. MacLachlan NJ, Kennedy PC: Tumors of the genital systems. *In: Tumors in Domestic Animals*, ed. Meuten DJ, 4th ed. pp. 561-567. Iowa State Press, Ames, IA, 2002

7. Sorscher S: Elevated parathyroid hormone-related peptide in a patient with an extragonadal germ-cell tumour and hypercalcemia. *Can J Surg* **47**:144, 2004

8. Veeramachaneni DN, Sawyer HR: Carcinoma in situ and seminoma in equine testis. *Apmis* **106**:183-185; discussion 185-186, 1998

9. Woodward PJ, Heindenreich A, Looijenga LH,

Oosterhuis JW, McLeod DG, Moller H, Manivel JC, Mostofi FK, Hailermaria S, Parkinson MC, Grigor K, True L, Jacobsen GK, Oliver TD, Talerman A, Kaplan GW, Ulbright TM, Sesterhenn IA, Rushton HG, Michael H, Reuter VE: Germ cell tumours. *In: World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs*, eds. Eble JN, Sauter G, Epstein JI Sesterhenn IA, pp. 221-249. IARC Press, Lyon, 2004



CASE II – 06-0534 (AFIP 3104113)

Signalment: 15-year-old, female, Manx cat, Felis domesticus

History: Two liver masses (4-6 cm in diameter) were discovered during a regular visit to the local vet. The cat was clinically healthy.

Gross Pathology: None reported.

Histopathologic Description: Sections of liver were partially effaced by a well-demarcated and unencapsulated mass composed of histologically normal adipose tissue and different cells of hematopoietic series. Cellular atypia and mitoses were not significant.

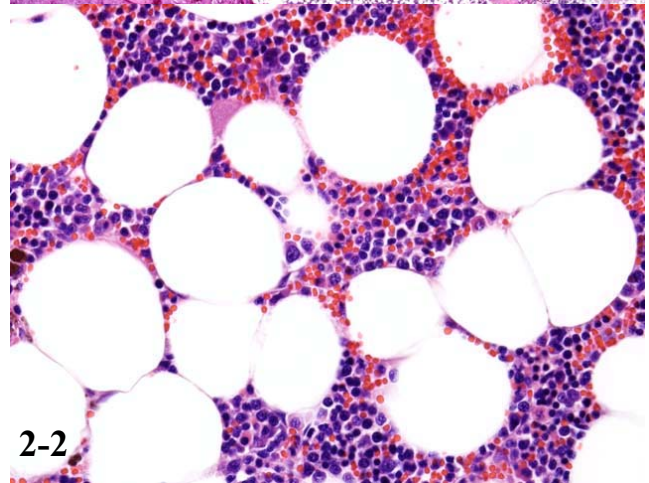
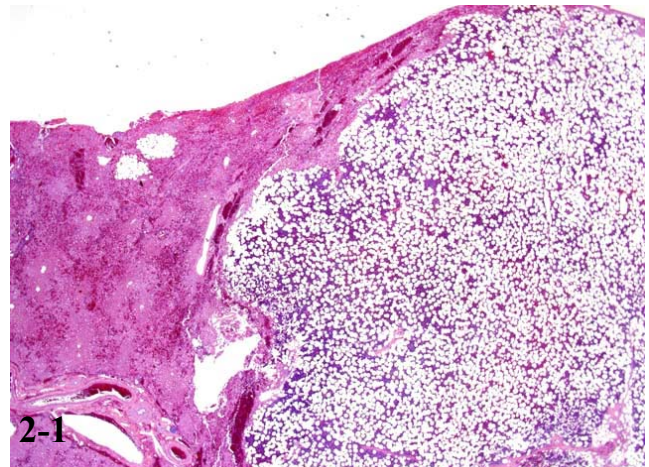
Contributor's Morphologic Diagnosis: Myelolipoma, liver

Contributor's Comment: Myelolipomas are rare, benign, and endocrinologically inactive tumors composed histologically of adipocytes with variable hematopoietic cells, including both mature and immature cells of the granulocytic, erythrocytic, and megakaryocytic series. They have been reported rarely in veterinary literature, particularly in the liver of cats and wild felidae; and in the spleen, adrenal gland, and spinal cord of dogs.(1,2) The vast majority of myelolipomas in humans occur within the adrenal glands, but extra-adrenal myelolipomas have been reported.(1) Feline hepatic myelolipomas are usually of no clinical significance, and they are usually diagnosed incidentally at necropsy during exploratory laparotomy or during abdominal ultrasonography. Metastasis has not been reported.(2) The pathogenesis of myelolipoma remains speculative. Theories concerning the pathogenesis include autonomous proliferation of bone marrow cells transferred during embryogenesis or metaplasia of certain mesenchymal cells triggered by various stimuli, including necrosis, infection, or stress.

When considering the differential diagnosis of hepatic myelolipomas, extramedullary proliferations of hematopoietic elements (EMH) is the primary differential. Myelolipomas are well-demarcated, discrete, single or multiple masses in any part of the liver; whereas, EMH appears as multifocal microscopic aggregates of hematopoietic cells in the perisinusoidal compartment and in portal areas. Also, hepatic EMH is usually not associated with forming discrete masses; however, severe EMH can lead to diffuse organ enlargement (hepatomegaly).

AFIP Diagnosis: Liver: Myelolipoma, multiple

Conference Comment: Myelolipomas have also been found in the adrenal glands of cattle, non-human primates, and sporadically in other various species. Histologically, these benign lesions are similar to those in the liver and are composed of both myeloid and erythroid hematopoietic tissue admixed with well-differentiated



2-1. Liver, cat. Myelolipoma. Focally, replacing and expanding normal liver architecture and compressing adjacent hepatocytes is a well circumscribed neoplasm composed of high numbers of adipocytes admixed with normal bone marrow elements. (HE 20X).

2-2. Liver, cat. Myelolipoma. The neoplasm is composed of numerous adipocytes admixed with myeloid and erythroid precursors. (HE 400X)

adipocytes. Bone formation and areas of mineralization have also been observed in these tumors. Rare cases of splenic myelolipomas have also been reported. Grossly, these lesions stand out from the splenic parenchyma as a focal area of pallor. The speculative pathogenesis of this lesion is metaplastic transformation of a resident cell population regardless of location (adrenal gland, liver, and spleen).(3)

Contributing Institution: Egyptian Society of Comp. and Clinical Pathology, Department of Veterinary Pathology, Alexandria University, Alexandria-EGYPT

References:

1. Al-Rukibat RK, Bani Ismail ZA: Unusual presentation of splenic myelolipoma in a dog. *Can Vet J.* 47(11):1112-4. 2006
2. Capen CC: Endocrine glands. *In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, ed. Maxie MG, 5th ed., vol. 3, pp.415. Elsevier Limited, St. Louis, MO, 2007
3. Stalker MJ, Hayes MA: Liver and biliary system. *In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, ed. Maxie MG, 5th ed., vol. 2, pp. 386. Elsevier Limited, St. Louis, MO, 2007
4. Valli VEO: Hematopoietic system. *In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, ed. Maxie MG, 5th ed., vol. 3, pp. 289. Elsevier Limited, St. Louis, MO, 2007

CASE III – CRL 2008-2 (AFIP 3104060)

Signalment: Domestic rabbit (*Oryctolagus cuniculus*), male, age and strain unknown

History: Clinically normal animal, incidental finding at necropsy

Gross Pathology: Occasional white to pale gray linear foci (0.5-1 cm diameter) in the left lateral lobe of the liver.

Laboratory Results: *E. cuniculi* seropositive

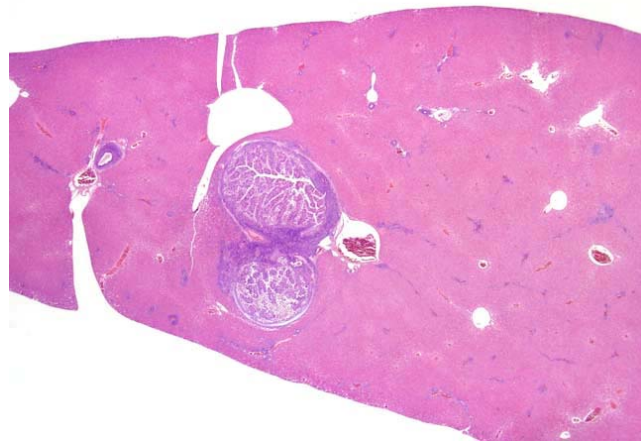
Histopathologic Description: Most portal areas and bile ducts have an infiltrate of lymphocytes, plasma cells, histiocytes and occasional heterophils. More severely affected regions have portal fibrosis, bile duct hyperplasia, and extension of the inflammation into the adjacent hepatic parenchyma. Occasionally there is marked dilation of bile ducts, with hyperplastic biliary epithelium thrown into papillary folds (**Fig. 3-1**), and large numbers of coccidial forms in various stages of development within the biliary epithelium and free in the lumen (**Fig. 3-2**).

Contributor's Morphologic Diagnosis: Liver: Cholangiohepatitis, chronic, nonsuppurative, multifocal,

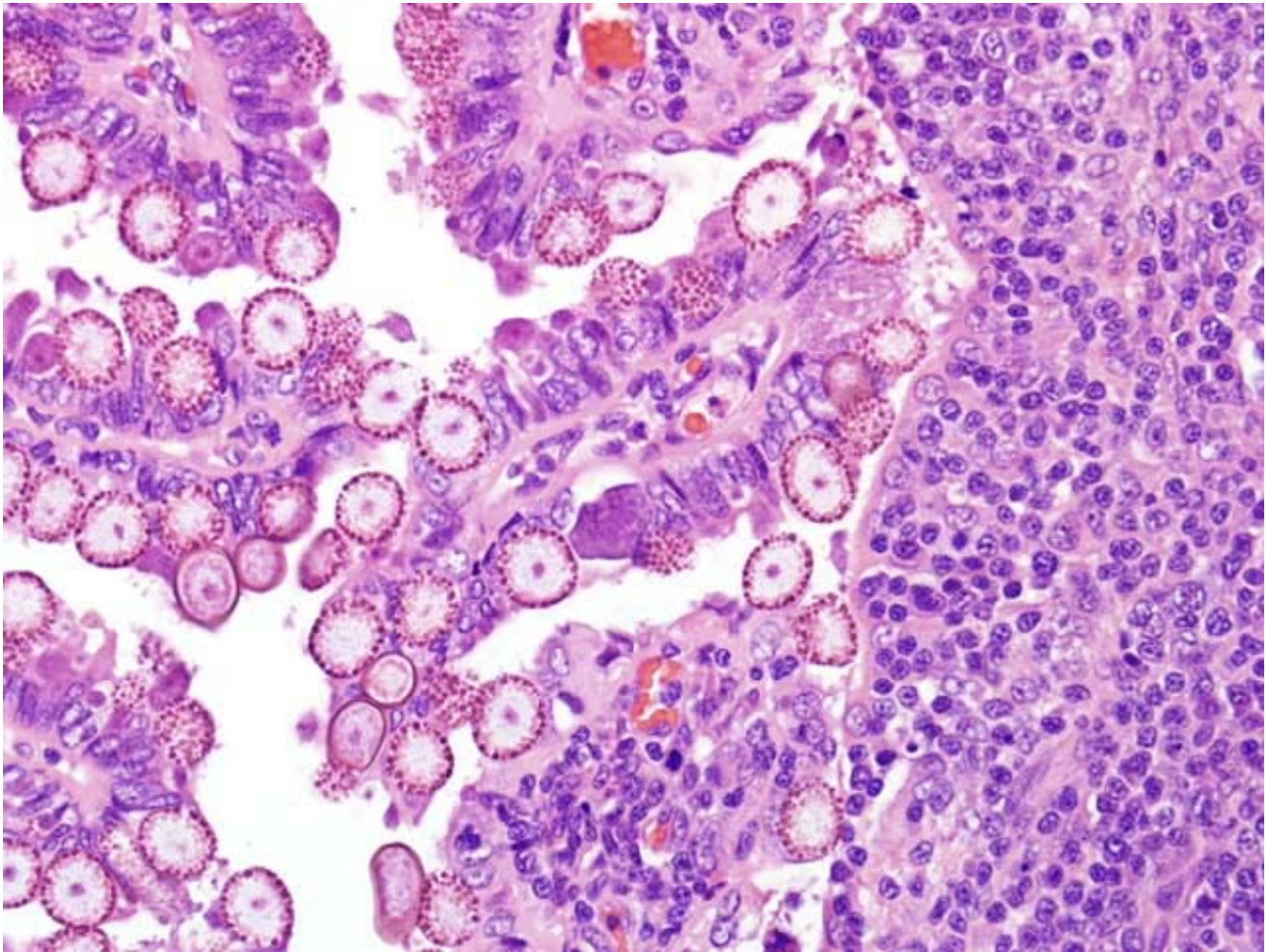
moderate, with bile duct proliferation and intralesional coccidia (*Eimeria stiedae*)

Contributor's Comment: *Eimeria stiedae* was identified using fecal centrifugation concentration.

The life cycle of *E. stiedae* is typical of *Eimeria* spp, in that all *Eimeria* are host-specific and have a direct life cycle.(2) Oocysts are not infective until sporulation, so ingestion of cecotroph feces does not result in autoinfection. Ingestion of sporulated oocysts (sporocysts) results in release (excystation) of sporozoites in the duodenum. Sporozoites invade the intestinal mucosa, are carried to the liver in the portal veins and/or lymphatics where they enter biliary epithelial cells and multiply asexually by schizogony.(5) Developing schizonts containing merozoites are evident within 3-6 days following infection, and gametogony can be identified eleven days post infection. In gametogony, the final generation merozoites form either macrogametes (female) or microgametes (male). After fertilization, macrogametes develop into oocysts, and enter the intestine through the bile, pass out of the host in the feces, and undergo sporulation. The prepatent period is 14-18 days, and oocysts may be shed in the feces for up to 7 or more weeks. Oocysts of *E. stiedae* contain 4 sporocysts, each of which contains two sporozoites. They are ovoid to elliptical, 28-42 um by 16-25 um with a micropyle. Sporocysts are 8-10 by 17-18 um and contain a Stieda body.(5)



3-1. Liver, rabbit. Multifocally, the bile ducts are markedly dilated and the lumina are filled with hyperplastic biliary epithelium which lines papillary projections. Bile ducts and portal areas are diffusely surrounded by a cellular infiltrate. (HE 20X).



3-2. Liver, rabbit. Diffusely within the hyperplastic biliary epithelium there are numerous protozoa in varying stages of development. (HE 400X).

Eimeria stiedae is common in domestic rabbits throughout the world, as well as cottontail rabbits and hares.(3) It is an important cause of morbidity and mortality in commercial rabbitries (the source of this rabbit) but is rarely seen in laboratory rabbits raised according to strict barrier procedures. *E. stiedae* infections may be subclinical or manifest as clinical disease, with occasional mortality. Weanling rabbits are most often affected (4) and may exhibit anorexia, lethargy, diarrhea, abdominal enlargement due to hepatomegaly, and icterus.

At necropsy, the liver contains variable numbers of raised, linear, bosselated, yellow to gray circumscribed lesions scattered throughout the hepatic parenchyma.(4) In severe cases there is hepatomegaly, with the liver

comprising up to 20% of body weight.(5) Microscopically there is marked dilation of bile ducts, extensive portal fibrosis, and a mixed inflammatory cell infiltrate in the portal zones. In affected bile ducts, there is hyperplasia of epithelium with papillary projections, with large numbers of gametocytes and oocysts typically present in affected ducts. The characteristic histologic findings of proliferative biliary changes and coccidial organisms are essentially diagnostic for this disease.

Changes in serum chemistry seen during the acute and convalescent stages of the disease indicate significant metabolic aberrations.(4) There is some evidence that rabbits heavily infected with *E. stiedae* may have an impaired immune response.

Table 3-1 Common *Eimeria*, *Isospora* and *Cystoisospora* spp. found in domestic animals. (5)

Animal	Coccidia	Organ affected
Cattle	<i>E. zuernii</i>	Distal small intestine
	<i>E. bovis</i>	Distal small intestine
Sheep	<i>E. ovinoidalis</i>	Terminal ileum/ cecum and colon
	<i>E. ashata</i>	Distal small intestine
	<i>E. bakuensis</i>	Distal small intestine
	<i>E. crandallis</i>	Distal small intestine
Goats	<i>E. ninakohlyakimovae</i>	Cecum and colon
	<i>E. caprina</i>	Cecum and colon
	<i>E. christensenii</i>	Distal small intestine
Swine	<i>Isospora suis</i> (neonatal pigs)	Distal small intestine
	<i>E. scabra</i> (weaners, growers)	Distal small intestine
	<i>E. deblickei</i> (weaners, growers)	Distal small intestine
	<i>E. spinosa</i> (weaners, growers)	Distal small intestine
Equine	<i>E. leuckarti</i>	Small intestine
Dogs	<i>C. canis</i>	Distal small intestine, large intestine
	<i>C. ohioensis</i> complex = (<i>C. burrowsi</i> , <i>C. ohioensis</i> , <i>C. neorivolta</i>)	Distal small intestine, large intestine
Cats	<i>C. felis</i>	Small intestine, large intestine
	<i>C. rivolta</i>	Small intestine, large intestine
Chickens	<i>E. acervulina</i>	Small intestine
	<i>E. necatrix</i>	Small intestine
	<i>E. tenella</i>	Ceca

AFIP Diagnosis: Liver: Cholangiohepatitis, proliferative, lymphoplasmacytic, chronic, multifocal, moderate, with intraepithelial coccidia (*Eimeria stiedae*)

Conference Comment: Coccidia are in the phylum Apicomplexa and are single cell, protozoal parasites. Members of the genus *Eimeria* and *Isospora* are generally host and organ specific, with lesions usually occurring in the gastrointestinal tract. These organisms also commonly infect young animals. **Table 3 -1** is a brief, non-comprehensive list of common *Eimeria* and *Isospora* found in domestic animals. In dogs and cats, the coccidia of importance are in the genus *Cystoisospora*.(5)

Contributing Institution: Charles River;
www.criver.com

References:

1. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp.260-270. Elsevier Limited, St. Louis, MO, 2007
2. Gardiner CH, Fayer R, Dubey JP: An Atlas of Protozoan Parasites in Animal Tissues, 1st ed., pp 20-30. USDA Agricultural Research Service Agricultural Handbook Number 651, 1988
3. Levine, ND: Veterinary Protozoology, pp178. Ames, IA, Iowa State University Press, 1985
4. Percy DH, Barthold SW: Pathology of Laboratory Rodents and Rabbits, pp 288-290. Ames, IA, Blackwell Publishing, 2007
5. Schoeb TR, Cartner SC, Baker RA, Gerrity LW: Parasites of rabbits. In: Flynn's Parasites of Laboratory Animals, ed. Baker DG, 2nd ed., pp. 454-457. Blackwell Publishing, Oxford, UK, 2007



CASE IV –N0710576 (AFIP 3105527)

Signalment: 17-month-old, male, PLB-23 rat (*Rattus norvegicus*)

History: The rat is part of a research colony maintained at our research institution and was singly housed in standard rat caging. A husbandry technician noted this rat to be hunched, scruffy, lethargic and dehydrated. A large volume of blood was found in the cage with no evidence of a laceration or open wound—no obvious visible source of blood could be found grossly. The rat

was dyspneic, experiencing agonal breaths and was humanely euthanized.

Gross Pathology: There is a large mass in the area of the pituitary gland. A large blood clot is found to fill the entire nasal cavity.

Laboratory Results: Microcytic, normochromic anemia, hyperglycemia, elevated liver enzymes, hyperphosphatemia and hyperkalemia. The decreased hematocrit and hemoglobin concentration are secondary to blood loss. The elevation in liver enzymes AlkP, ALT, and AST may be secondary to hypoxia or the elevated blood glucose (diabetes mellitus). The increased potassium and phosphorus are unexplained and may be due to decreased renal clearance, dehydration/shock, or tissue trauma. In addition, the blood sample was slightly hemolyzed, which may account for the elevated potassium, phosphorous and elevated liver enzymes. See detailed bloodwork results in **Table 4-1**.

Histopathologic Description: There is a large multinodular mass (~2 cm wide) that extends from the base of the brain in the area of the pars distalis posteriorly into the cerebellum, dorsally into the overlying cerebrum and cerebellum. In addition, there is a spatially distinct section of tumor present unilaterally in the lateral ventricle in the area of the hippocampus. The neoplastic cells are arranged in solid sheets to compact cords with a fine fibrovascular stroma. There are numerous small to large cystic spaces filled with eosinophilic proteinaceous material admixed with erythrocytes. The neoplastic cells are irregularly round and contain a single predominantly vesiculate nucleus with a single prominent nucleolus and an amphophilic to slightly eosinophilic cytoplasm (**Fig. 4-1**). Nuclear pleomorphism is high. Occasionally larger bizarre forms are observed. The third and lateral ventricles are distended and contain fibrin, edema and hemorrhage. The neoplastic cells are periodic acid-Schiff negative.

The nasal pharynx and nasal cavity are full of blood admixed with fibrin. At the level of the eyes, nasal cavity and upper molars, there is a unilateral focal area where one molar and the adjacent tissue are altered. There is a single large hair (whisker) and other cross sections of hair. The large hair extends from within the oral cavity along the medial aspect of one molar sulcus and is embedded into the overlying soft tissues of the palate. Surrounding the larger piece of hair there is a thin layer of stratified squamous epithelium and beyond this there is abundant fibrous connective tissue admixed with neutrophils, macrophages, and scattered lymphocytes and plasma cells. At the margin of the oral cavity there are

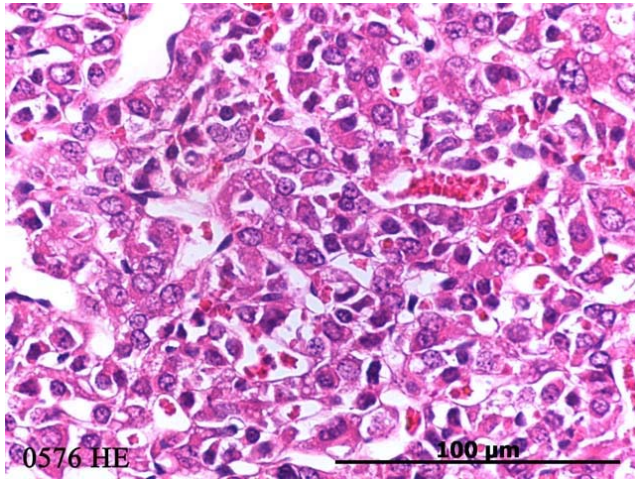
Table 4-1.

Complete Blood Count	Results	Reference Range (Units)	Serum Biochemistry Panel	Results	Reference Range (Units)
Hemoglobin	10.4 L	11.4-19.2 g/dL	Glucose	465 H	60-125 mg/dL
Hematocrit	28.0 L	33-50 %	Urea Nitrogen	22	9-30 mg/dL
WBC	6.8	5.5-11 x10 ³ /uL	Creatinine	0.5	0.4-1.0 mg/dL
RBC	5.53	5.5-10.5 x10 ⁶ /uL	Total protein	6.4	4.5-6.5 g/dL
MCV	51	Fl	Albumin	3.2	2.0-6.2 g/dL
MCH	18.8	Pg	Total bilirubin	0.1	0-1 mg/dL
MCHC	37.1	g/dL	Alkaline Phosphatase	91 H	15-45 U/L
Platelet Count	634	x10 ³ /uL	ALT	69 H	10-35 U/L
Platelet Estimate	Increased		AST	177 H	10-45 U/L
<i>Differential</i>		<i>Units</i>	Cholesterol	122	50-250 mg/dL
Neutrophils	4148	61%	Calcium	12.0	8-12 mg/dL
Bands	0		Phosphorous	10.8 H	4.2-8.5 mg/dL
Lymphocytes	2448	36%	Sodium	142	140-160 mEq/L
Monocytes	136	2%	Potassium	7.1 H	4.3-5.8 mEq/L
Eosinophils	68	1%	Chloride	93	90-110 mEq/L
Basophils	68	1%	Albumin/ Globulin Ratio	1.0	0.4-1.1
Polychromasia	Slight		BUN/Creat Ratio	44	
Prothrombin Time	13.1	Secs	Globulin	3.2	2.5-4.8
PTT	14.3	Secs	CPK	241	U/L
Fibrinogen	262	Mg/dL			
D-Dimer	< 250	Ng/mL			

greater numbers of neutrophils. There are similar inflammatory cells surrounding the smaller sections of hair. There is marked loss of bone in the subjacent maxilla. The adjacent molar occlusal surface shape is altered with the lingual aspect longer and curved toward the buccal side. There is loss of the roots and intervening bone with no visible pulp cavity. There is an inflammatory infiltrate comprised predominantly of neutrophils at the base of the root. In the ipsilateral buccal mucous tunic to the affected molar, the oral mucosa is absent and the underlying lamina propria is acutely necrotic with a large amount of fibrin and

hemorrhage admixed with blood.

While the pituitary mass was considerable in size, it appears to have been an incidental finding in this animal, as the cause of impending death was respiratory failure resulting from the large blood clot within the nasal cavity. This can occur in rats because the anatomy of the oronasal cavity of rats is unique rendering them obligate nose-breathers. In rats, the soft palate is long, and the anterior opening of the esophagus, the epiglottis, and the larynx lies anterior to the nasopharyngeal opening. Therefore, an obstruction in this area would prevent a rat



4-1. Pituitary gland, rat. The neoplasm is composed of polygonal cells supported by a fine fibrovascular stroma; neoplastic cells have variably distinct cell borders with moderate amounts of eosinophilic finely granular cytoplasm and irregularly round to oval nuclei with finely stippled chromatin and generally one variably distinct nucleolus. (HE 400X). Photomicrograph courtesy of Section of Comparative Medicine, School of Medicine, Yale University New Haven, CT.

from being able to breathe.

Contributor’s Morphologic Diagnosis: Pituitary Par
Distalis: Carcinoma, chromophobic

Contributor’s Comment: Pituitary tumors occur in most animal species, but occur rather frequently in laboratory rats and dogs. The classification of pituitary tumors as chromophobic, acidophilic and basophilic is based on the histologic staining characteristics of the granules they contain.(3) This traditional classification scheme is still in use, but does not categorize the functionality of the tumor and is therefore moving out of

favor. Immunohistochemical demonstration of the various types of pituitary hormones contained within the tumor is another method of classification of these neoplasms and may also aid in diagnosis and prognosis. (5) The following table, extracted from Jones, et. al., demonstrates the hormones secreted and typical staining patterns of the cells of the anterior pituitary.

Most pituitary tumors are adenomas and grow by expansion thereby creating a space-occupying lesion potentially interfering with the normal function of the cells within the pituitary, hypothalamus, thalamus and other surrounding structures. The clinical signs associated with the lesion are often linked to which types of hormones are secreted. Although they occur

Table 4-2. Endocrine cells and hormones of the anterior pituitary (3)

Cell type	Hormone	Cell characteristics
Somatotroph (type 2 acidophil)	Growth Hormone	H&E: acidophilic granules PAS: negative EM: abundant, dense granules 350nm
Lactotroph (type 1 acidophil)	Prolactin	H&E: acidophilic or chromophobic granules PAS: negative EM: sparse, dense granules 600-900nm
Gonadotroph (type 2 basophil)	Follicle-stimulating Hormone Leutinizing Hormone	H&E: basophilic PAS: positive EM: dense granules 200-250nm
Thyrotroph (type 1 basophil)	Thyroid-stimulating Hormone	H&E: basophilic PAS: positive EM: dense granules 150nm
Corticotroph (type 3 basophil)	Adrenocorticotropic Hormone	H&E: basophilic PAS: weakly positive EM: variably dense granules 200-400nm Cytoplasmic filaments
Melanotroph	Melanocyte-stimulating Hormone	H&E: basophilic PAS: positive

infrequently, metastatic pituitary neoplasms have been reported in a variety of species. These lesions can produce destructive effects on the pituitary, hypothalamus and thalamus leading to a multitude of clinical signs.(3)

Even though pituitary carcinomas occur with much less frequency than pituitary adenomas in most rats, in this case the tumor invasion into the overlying brain suggests that this tumor is a carcinoma. In addition, the fact that the tumor cells had high pleomorphism with bizarre forms suggests carcinoma.

In a study by McComb, et al (1984), it was found that in rats over 24 months of age, pituitary adenomas were found in 85% of male and 79% of female SD rats. Of these tumors, 47% were prolactin (PRL)-containing and 16% were luteinizing hormone (LH)-containing adenomas. The remaining 37% were made up of tumors containing thyroid-stimulating hormone (TSH), growth hormone (GH), adrenocorticotrophic hormone (ACTH) or some combination thereof, as well as immunonegative adenomas.(5) In another study done by Nagatani, et al (1987), 736 rats of various inbred strains ranging from 13 to 24 months of age were screened for pituitary tumors. Pituitary tumors were found in 284 of the 736 rats, with some rats having more than one lesion.(3) In addition to spontaneously occurring pituitary tumors, chronic estrogen treatment can induce prolactin-secreting tumor growth in the anterior pituitary of Fischer 344 rats.(8)

In dogs, ACTH-secreting tumors are the most common of the functional pituitary tumors. While these tumors can arise from the pars intermedia or the pars distalis, most commonly they are chromophobic adenomas composed of either large or small cells arising from the pars distalis. ACTH-secreting tumors frequently result in the development of adrenocortical hyperplasia and hyperfunction and cause pituitary-dependent Cushing's disease.(3)

While cats are not considered a species that commonly develops pituitary tumors, one study found that 16 out of 16 diabetic cats with insulin resistance also had pituitary adenomas manifesting as acromegaly or hyperadrenocorticism.(2)

The most frequently occurring pituitary neoplasm in horses is the adenoma of the pars intermedia leading to a variety of clinical signs including hirsutism, polyphagia, muscle wasting, hyperglycemia, and diabetes insipidus, among others.(1)

In humans, pituitary neoplasms represent approximately 10% of the intracranial tumors, the most common of

which are prolactin secreting pituitary adenomas.(3,5,4) Spontaneous pituitary adenomas have also been described in parakeets and mice.(5)

AFIP Diagnosis: Pituitary gland, pars distalis: Adenoma

Conference Comment: Conference participants debated the differentials of adenoma and carcinoma for this case. Conference participants did not observe invasion of the overlying brain in their tissue sections. Therefore we prefer the diagnosis of adenoma based on the lack of tissue or vascular invasion and the low mitotic rate. A moderate degree of anisocytosis and anisokaryosis does not preclude the diagnosis. The AFIP Department of Neuropathology concurred with this conclusion. The neoplasm is further classified as a lactotroph adenoma based on immunohistochemical procedures performed at AFIP; the neoplasm is positive for prolactin and negative for ACTH, FSH, GH, LH, and TSH.

Contributing Institution: Section of Comparative Medicine, School of Medicine, Yale University
New Haven, CT

References:

1. Capen, CC: Endocrine glands. *In: Jubb, Kennedy, and Palmers' Pathology of Domestic Animals*, 5th ed., pp. 339-346. Saunders Elsevier, Philadelphia, PA, 2007.
2. Elliot DA, Feldman EC, Koblik PD, Samii VF, Nelson RW: Prevalence of pituitary tumors among diabetic cats with insulin resistance. *JAVMA*, 216 (11): p. 1765-1768, 2000
3. Jones TC, Hunt RD and King NW: *Veterinary Pathology*, 6th ed., Williams & Wilkins, Baltimore, MD, pp. 1224-1232, 1997
4. McComb DJ, Hellmann P, Kovacs K, Scott D, Evans WS, Burdman JA, and Thorner MO: Spontaneous sparsely-granulated prolactin-producing pituitary adenomas in aging rats: *Neuroendocrinology*, 41: 201-211, 1985
5. McComb DJ, Kovacs K, Beri J, and Zak F: Pituitary adenomas in old sprague-dawley rats: a histologic, ultrastructural, and immunocytochemical study. *JNCI*, 19(5): p 1143-1157, 1973
6. Nagatani M, Miura K, Tsuchitani M, Narama I: Relationship Between Cellular Morphology and Immunocytological Findings of Spontaneous Pituitary Tumours in the Aged Rat. *J Comp Path*, 97: 11-20, 1987
7. Percy DH, Barthold SW: *Pathology of Laboratory Rodents and Rabbits*. 3rd ed., pp.108, 122, 173-174, 306. Blackwell Publishing, Ames, IA, 2007
8. Wendell DL, Platts A, Land S: Global Analysis of Gene Expression in the Estrogen Induced Pituitary

Tumor of the F344 Rat. *J Steroid Biochem & Mol
Biology* 101: 188-196, 2006



Notes: