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CASE I – H05-0326 (AFIP 2983869)

Signalment: Seven month old, mixed sex, *Cairina moschata*, Muscovy duck

History: An estimated 160 out of 500 commercial 2 to 4 month old Muscovy ducks presented with green tinged diarrhea, inappetence, polydipsia, fever, and leg weakness over a period of 17 days. A total of 48 ducks died. Other poultry and other age groups of ducks on the property were not affected (1).

Gross Pathology: All birds examined had similar lesions. There were multiple circular pale cream foci 2 to 3 mm diameter randomly scattered throughout the parenchyma of the liver and spleen. There was marked splenomegaly. The distal colon and rectum contained abundant green tinged liquid (1).

Histopathologic Description: Liver and spleen (H&E). There is randomly distributed multifocal to locally extensive well demarcated coagulative necrosis with an infiltrate of granulocytes and macrophages, and mineralization. Liver and spleen (Warthin-Starry). Areas of coagulative necrosis contain occasional groups of spirochetes.

Contributor’s Morphologic Diagnosis: Liver and spleen: Subacute marked multifocal to locally extensive coagulative necrosis and caseogranulomatous hepatitis / splenitis with mineralization. Liver and Spleen (Warthin-Starry). Coagulative necrosis with spirochetes (consistent with *Borrelia anserina*).

Contributor’s Comment: Avian borreliosis (avian spirochetosis) affects a wide range of bird species. The causative organism, *Borrelia anserina*, is an actively motile coiled spirochete 0.25 x 8 – 20 µm that can be propagated in duck or...
chicken embryos or chicks. Occurrence is worldwide, but reflects the temperate and tropical range of the vector *Argas persicus* (the cosmopolitan fowl tick), or other *Argas* spp. An infected Argas tick transmits *Borrelia anserina* to the avian host at every feeding, resulting in an acute systemic bacterial infection. There are many strains of *Borrelia anserina* with diverse serotypes and variable virulence. Young birds are more susceptible than adults. The disease course is one to two weeks and severity ranges from mild to fatal. Gross lesions range from splenomegaly with petechiae or ecchymoses, to multifocal necrosis. Spirochetes may be demonstrated in areas of caseous necrosis by Warthin-Starry stain. Antemortem diagnosis is by demonstrating spirochetes in Giemsa stained blood smears. Young birds develop a massive bacteremia that persists for several days. In adult birds the bacteremia is minimal and transient. Serologic diagnosis is problematic due to the existence of diverse serotypes (2).

**AFIP Diagnoses:**

1. Liver: Necrosis, coagulative, multifocal, with fibrin thrombi, Muscovy duck (*Cairina moschata*), avian.
2. Spleen: Granulomas, multifocal and coalescing, with mineralization.

**Conference Comment:** The contributor provides an informative review of avian borreliosis. Attendees discussed the differential diagnoses for well-circumscribed foci of coagulative necrosis in the liver. (e.g. in cattle, *Fusobacterium necrophorum*; in turkeys, *Histomonas meleagridis*; in reptiles, *Entamoeba invadens*; and primates, *E. histolytica*). With the aid of Warthin-Starry 4.0 tissue stain, *Borrelia* spp. spirochetes became readily identifiable within areas of coagulative necrosis.

Other important veterinary diseases caused by *Borrelia* spp. include: Lyme disease due to infection with *Borrelia burgdorferi*; bovine borreliosis caused by *Borrelia theileri*; porcine spirochetosis due to *Borrelia suilla* (ulcerative dermatitis); and ulcerative gingivitis or trenchmouth” in humans, non-human primates, and rarely puppies, caused by *Borrelia vincentii* (3).

Ixodes deer ticks transmit *B. burgdorferi* to dogs. In dogs, Lyme disease is characterized by six to eight weeks of sudden onset anorexia, vomiting, lethargy, and weight loss with or without a concurrent or recent history of lameness. Some dogs will develop signs of acute, progressive renal failure (i.e. uremia, azotemia, proteinuria, peripheral edema and body cavity effusions). The characteristic microscopic renal lesions associated with Lyme disease are membranoproliferative glomerulonephritis, lymphoplasmacytic interstitial nephritis and renal tubular degeneration, necrosis and regeneration (4).
In humans, Lyme disease affects multiple organ systems and can result in skin lesions, fever and lymphadenopathy followed by joint and muscle pain, cardiac arrhythmias, meningitis, chronic arthritis and encephalitis. Also in humans, relapsing fever, caused by *Borrelia recurrentis* and transmitted by soft ticks or lice, is characterized by chills, fever, headache and fatigue, followed by disseminated intravascular coagulation and multiorgan failure (5).

*Borrelia* spp. evade the immune system and prevent recognition by host antibodies by varying or shedding their surface antigens or outer membrane proteins. In Lyme disease, as the antibody response to one surface protein is mounting, the bacteria expresses an alternate protein thereby escaping immune recognition (5).

Interestingly, *Borrelia coriaceae* has, until recently, been considered as a potential etiology for epizootic bovine abortion (EBA). A recently published journal article; however, identifies the etiologic agent as a deltaproteobacterium of the class *Proteobacteria*. The only other mammalian pathogen in this class is *Lawsonia intracellularis* (6).

The contributor notes that spirochetes are identifiable in Giemsa stained blood smears. Other organisms found in avian blood smears include Apicomplexan parasites such as:
- *Leucocytozoon* spp. (gamonts or gametes in blood smears identifiable with Wright or Giemsa stain)
- *Haemoproteus* spp. (schizonts in visceral endothelial cells; gametocytes develop in circulating erythrocytes; birefringent pigment granules in infected erythrocytes)
- *Plasmodium* spp. (avian malaria; schizogony in peripheral blood; gametocytes in mature erythrocytes, contain birefringent pigment granules known as malaria pigment)

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**References:**

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**CASE II – 105.126 (AFIP 2987114)**

**Signalment:** 7-year-old, castrated male, Ferret, *Mustela putorius furo*, ferret

**History:** Lethargy of unknown duration. Abdominal mass identified during ultrasound. Animal was euthanized.

**Gross Pathology:** A 5.5 x 7.0 x 4.0 cm soft to firm, tan/white to yellow, lobular, hemorrhagic and necrotic mass is present within the left caudal abdomen. This mass displaces adjacent abdominal viscera. The left adrenal gland is not identified. The right adrenal gland contains a 0.5 x 0.5 x 0.5 cm tan to yellow, soft, nodular cortical mass. Approximately 10 mls of sanguinous peritoneal fluid is present. The lungs appear grossly normal.

**Laboratory Results:** WBC: 3.800 x 10³ (normal 5.900–15.400)
Hematocrit: 25% (normal 36-51%)
BUN: 58 (normal 12–43)

No other significant CBC or chemistry abnormalities were identified.

**Histopathologic Description:** Within the abdomen, an expansile solid mass with prominent fibrous septae and regionally extensive areas of hemorrhage and necrosis are noted. Neoplastic cells form numerous cystic structures that contain a lightly basophilic to amphophilic staining acellular mucinous-type material. Neoplastic cells are cuboidal to polygonal with scant darkly eosinophilic staining cytoplasm and prominent nuclei that often contain one or more nucleoli. The cytoplasm of neoplastic cells often contains variably sized clear to wispy vacuoles. Anisocytosis and anisokaryosis are moderate. Approximately two mitotic figures are present per high powered field.
Several pulmonary blood vessels contain variably sized intraluminal bundles of metastatic neoplastic cells that are identical to the cells identified within the abdominal mass.

Contributor’s Morphologic Diagnoses:
1. Abdominal mass: Adrenal cortical carcinoma with myxoid differentiation
2. Lungs: Multifocal intravascular metastatic carcinoma
3. Right adrenal (not provided): Adrenal cortical carcinoma

Contributor’s Comment: Adrenal cortical carcinoma is the second most common neoplasia within the domestic ferret. Common clinical signs include vulvar enlargement, bilaterally symmetrical alopecia and polyuria/polydipsia. Clinical signs are consistent with overproduction of estrogenic steroids. Plasma cortisol levels are consistently within normal limits. Females are affected more commonly than males and a mean age of 5 years has been reported. Adrenal cortical carcinoma with myxoid differentiation is a variant of adrenal tumors that appears more malignant than typical well differentiated adrenal cortical carcinomas. The clinical signs in ferrets that have the myxoid variant are similar to those seen in non-myxoid adrenal carcinomas. Immunohistochemistry results in positive cytoplasmic staining for vimentin, synaptophysin and α-inhibin within neoplastic cells. This pattern of staining is indicative of a zona reticularis origin. The origin of the neoplastic cells is uncertain; however possible explanations include differentiation from neoplastic cells of the zona reticularis, degeneration of neoplastic cells and possible origination from ectopic rests of gonadal stromal granulosa or Sertoli cells. A similar neoplasm of adrenocortical myxoid variation is rarely observed within people. These tumors in people share similar clinical pathology including overproduction of sex hormones. The adrenal myxoid variant is also present in both carcinomas and adenomas of people, whereas it is only identified in the carcinomas of ferrets.

2. Lung, vascular lumina: Carcinoma, adrenal cortical, metastatic.

Conference Comment: In ferrets, adrenocortical carcinoma is second only to pancreatic islet cell tumors in frequency.

Four main differential diagnoses for a cellular proliferation of the adrenal glands includes (4):
- Adrenocortical carcinoma: identified microscopically by cellular atypia, invasion beyond the adrenal capsule and/or evidence of distant metastasis
• Cortical adenomas: do not extend beyond the capsule, are well-demarcated, and variably encapsulated
• Nodular hyperplasia: unencapsulated nodular proliferation confined to the adrenal cortex
• Pheochromocytoma

As noted by the contributor, this newly described variant of adrenocortical carcinoma is recognized microscopically as having multiple cystic spaces lined by neoplastic polygonal cells and containing abundant mucinous product. Neoplastic cells are positive for vimentin, synaptophysin and α-inhibin. Inhibins are peptide hormones produced by the granulosa cells in female ovarian follicles and by Sertoli cells in the male seminiferous tubules. They are selectively expressed by cells of sex cord stromal derivation. While anti-α-inhibin can be used to identify sex cord stromal tumors and trophoblastic tumors, it is also helpful to differentiate adrenocortical tumors.

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References:
CASE III – 203-06 (AFIP 2988627)

Signalment: 1 year old, male, intact ferret

History: Acute onset of lethargy with fever (107.8 F) that did not respond to antibiotic therapy. One day later exhibited signs suggesting abdominal pain. Two days after onset, feet were swollen, the fever persisted and lethargy was worse. No response to additional treatments (fluids and steroids). There was no improvement on day 3 and the ferret was euthanatized. The referring veterinary practitioner performed a necropsy and submitted fresh and fixed tissues for analysis.

Gross Pathology: There were no gross lesions identified in submitted fresh and fixed tissues. No gross lesions were observed at necropsy by the referring veterinarian. Bloodwork was not completed.

Laboratory Results: No bacteria were isolated using routine aerobic and anaerobic culturing of muscle, lung and liver. Virus isolation using several tissues on MDCK, A72-180, BHK-21, and bovine turbinate cells did not demonstrate viral pathogens.

Histopathologic Description: Skeletal muscle of the esophagus and hindlimb muscles: The primary change is a severe suppurative myositis. Granulocytes, mostly neutrophils, with few mononuclear leucocytes dissect around muscles within fascia and within muscles between muscle bundle and myofibers. Occasional myocytes are shrunken or swollen (necrotic) and infiltrated with neutrophils. Increased numbers of granulocytes line lumina of small blood vessels in the connective tissue surrounding the esophagus and muscles.

Similar, but less intense lesions were identified in sections of lumbar and forelimb muscles and right and left ventricular myocardium. Other minor changes included septal capillary neutrophilia in lung, splenic lymphoid nodular hyperplasia, hepatic lipidosis, and pigment in renal tubular epithelium. These were the only tissues examined microscopically.

Contributor’s Morphologic Diagnosis: Skeletal muscle, multiple sites; Inflammation, suppurative, acute, severe with occasional myonecrosis.

Contributor’s Comment: Disseminated Idiopathic Myositis (DIM) appears to be a newly recognized disease in ferrets that was first described clinically in 2003. Clinical signs include high fever, lethargy/weakness, reluctance to move, pain on handling, decreased appetite, increased respiratory and heart rate, nasal discharge, and enlarged lymph nodes. Microscopic lesions are as described above.
AFIP Diagnosis: Skeletal muscle, hindlimb (per contributor) and esophagus: Myositis, neutrophilic, acute, multifocal, with scattered myonecrosis, ferret, mustelid.

Conference Comment: Conference attendees agreed that the specificity of the neutrophils for the skeletal muscle of the esophagus is most likely immune-mediated. The inflammation is composed primarily of nondegenerate rather than degenerate neutrophils and the esophageal mucosa is completely spared. In other cases, non-muscular organs such as the brain, liver, lung (bronchopneumonia) and spleen (extramedullary hematopoeisis) have also been affected. As of the time of this write-up, additional information for this disease has still not been published.

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References:

CASE IV – B01361/D (AFIP 2988203)

Signalment: 7 year old, male, Rottweiler, Canis familiaris, dog.

History: The dog was presented for physical examination with a history of ascites, and right cardiac failure was diagnosed. Thoracic examination by radiography and echocardiography revealed a mass within the lumen of the right atrium. The patient underwent open-heart surgery with dissection and removal of a 5 cm diameter, bright red, cerebriform, soft mass from the interatrial septum. Microscopic examination of the lesion was performed. After 9 months, the dog was submitted again to cardiac surgery for a relapse of the mass within the lumen of the right atrium and main vessels of the heart base. The animal was suppressed during surgery.

Gross Pathology: The lesion evident during second surgery within the right atrium was a multinodular, whitish to tan brown, smooth, lardaceous and soft mass. The neoformation was attached to the inter-atrial wall and it was growing within the pulmonary arteries and the senum venosum lumen (Fig. 1, kindly provided by Dr. R. Bussadori).
Laboratory Results: Hematobiochemistry was not available to be submitted since the case was referred for a second opinion to the surgeon.

Histopathologic Description: The section is characterized by a poorly demarcated, irregular shaped, unencapsulated, densely cellular, infiltrative, multi-lobular mass. Fine septa and small disseminated capillaries are observable. The cells are discrete, round to polyhedral (15-20 mm diameter), and arranged in packets and short cords. Occasionally, cells form lacune filled with erythrocytes. The cytoplasm is scant to moderate, lightly eosinophilic, finely granular, often vacuolated and chromaffin granules are evident with Grimelius specific staining (Fig. 2). The nuclei are round to oval (10-12 mm diameter), usually central, vesicular with marginated chromatin and a single paracentral nucleolus is often seen. Anisokaryosis and anisocytosis are mild to moderate and mitotic figures are rare (< 1 x high power field). Small lymphocyte aggregates are rarely seen within the lesion. Multifocal necrosis and extensive hemorrhages are also present; the latter is associated with disseminated pigment laden macrophages. Diffuse signs of degeneration are evident, particularly in the infiltrative areas.

Contributor’s Morphologic Diagnosis: Intracardiac chromaffin paraganglioma.

Contributor’s Comment: A paraganglioma was diagnosed arising from the interatrial groove, as confirm also by immunohistochemistry: a diffuse cytoplasmic positivity with anti-NSE and anti-Chromogranin A was observed (Fig. 3-4).

Primary cardiac neoplasms are rare in domestic animals, with an incidence varying in dogs between 0.19% and 3.1%, and the heart is one of the rarest locations of paragangliomas. However, the latter are second to hemangiosarcomas among tumors affecting the heart of dogs (1, 2, 3, 4, 5). Paragangliomas are neuroendocrine neoplasms. They arise from paraganglionic tissue, which is a collection of neuroepithelial cells of the autonomic nervous system. These cells are of neural crest origin and are classified as chromaffin (catecholamine secreting) and non-chromaffin (non-catecholamine secreting). Paragangliomas arising from the adrenal medulla and the aorticosympathetic ganglia are innervated by the sympathetic nervous system and secrete catecholamines. They commonly occur in the adrenal medulla where they are referred as pheochromocytomas (1). In contrast, tumors from elsewhere generally do not secrete catecholamines and are innervated by parasympathetic fibers (1, 6). These nonchromaffin paragangliomas, also called chemodectomas, originate mainly from the aortic body in the thorax, carotid body in the neck, and the glomus jugulare of the middle ear. Aortic body tumors are the most frequent type in domestic animals and are also referred to as heart base tumors (2, 4). Paragangliomas are frequently benign, slow growing, and locally invasive. They are highly vascularized tumors that infiltrate the coronary circulation. The main feeding vessels typically originate from the left coronary
system. (2, 4, 7, 8) There are no proven histological criteria to differentiate benign from malignant cardiac paraganglioma and there is no direct correlation between tumor size and malignant potential.

The neoplasm found in the heart of this subject showed a strong cytoplasmic chromaffin reaction and therefore we classified it as an extra-adrenal intra-cardiac chromaffin paraganglioma. In human medicine, 95% of all reported chromaffin paragangliomas are in the adrenal medulla and only 2% are above the diaphragm, accounting for 0.3% of all mediastinum neoplasms. In the mediastinum, 75% are found in the posterior compartment. Less than 50% are functional (1, 7, 8). The middle compartment contains only a few small clusters of chromaffin tissue that could potentially provide a site for tumor development. These neuro-endocrine cell nests are located close to the sympathetic fibers of the middle mediastinum or they could be considered as ectopic chromaffin tissue (1, 7, 9).

In our opinion, this is a very rare location for paraganglioma in domestic animals. Less than 50 cases of cardiac paragangliomas are described in human medicine (10). They can involve the left atrial wall, left ventricle and, less commonly the inter-atrial septum. More rarely they can originate in the right atrium (1, 9). These tumors are locally invasive and can produce symptoms secondary to pericardial involvement or through invasion of the conduction system (8).

AFIP Diagnosis: Heart, right atrium (per contributor): Cardiac paraganglioma, Rottweiler, canine.

Conference Comment: This case prompted the review of the distribution and classification of normal paraganglia as well as the appropriate name for neoplasms having typical neuroendocrine or “zellballen” histologic features which arise in or near the heart base.

Paraganglia are neuroendocrine organs composed of neuroendocrine cells and supporting (sustentacular) cells. They are of neural crest origin. Paraganglia are anatomically widely dispersed, but can be broadly divided into two groups. The sympathetic paraganglia are distributed along the sympathetic chains in the para-axial regions of the trunk. The most familiar example of a sympathetic paraganglion is the adrenal medulla. Neuroendocrine neoplasms arising in the adrenal medulla are called pheochromocytomas. Neoplasms arising from sympathetic paraganglia in other sites have been called chromaffin paragangliomas or extra-adrenal pheochromocytomas.
The second group is the parasympathetic paraganglia, distributed along and innervated almost exclusively by parasympathetic nerves of the cranial and thoracic branches of the glossopharyngeal and vagus nerves. Examples of this type are the chemoreceptor organs, including the aortic and carotid bodies. Neoplasms arising from these organs have been called aortic or carotid body tumors, chemoreceptor tumors, chemodectomas, or nonchromaffin paragangliomas. In veterinary medicine, neoplasms of chemoreceptor origin occur primarily in dogs, with infrequent occurrence in cats and cattle. Brachycephalic breeds like the Boxer and Boston Terrier are predisposed.

Conference attendees agreed that the neoplasm has the typical features of a neuroendocrine tumor and an infiltrative growth pattern. However, because there are no consistent histologic differences between paragangliomas of sympathetic origin and those of parasympathetic origin, we are unable to classify this tumor further based on the hematoxylin and eosin section. The contributor’s comments on the significance of a positive chromaffin reaction are thought provoking. In most references, the term chromaffin paraganglioma denotes a tumor of sympathetic paraganglion origin which produces catecholamines. Chromaffin positivity was based on identification of cytoplasmic granules which stained with the Grimelius stain. In our lab, the neoplastic cells are positive with the Churukian-Schenk stain, which, like the contributor’s Grimelius stain, is another argyrophil stain. However, the neoplastic cells are negative with Fontana-Masson stain, an argentaffin stain, a finding that suggests this may be a nonchromaffin paraganglioma. Additionally, all paragangliomas may produce catecholamines to some extent, detectable by methods more sensitive than the chromaffin reaction (11). In some cases, the chromaffin reaction may not reliably separate these tumors (12). Both types can be positive for the immunohistochemical markers neuron specific enolase, chromogranin A and synaptophysin. In the present case we cannot definitively rule out chemodectoma in this anatomic location, so we prefer the more inclusive diagnosis of cardiac paraganglioma.

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References:

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