CASE I: 59688 (AFIP 3134335).

Signalment: Multiple adult long-tailed finches (Poephila acuticauda).

History: Tissues submitted were from multiple individuals that were among 12 captive Australian finches in the same exhibit that presented with feather loss, dehydration, weight loss, lethargy, and/or respiratory distress. Most birds died or were euthanized shortly after presentation (1-2 days). On physical exam, these birds were very thin and dehydrated, and had feather loss on their heads and necks. Many were treated with fluids, enrofloxacin, and injectable calcium with little clinical response. Other affected species included the diamond firetail finch (Emblema guttata), star finch (Neochmia ruficauda), black-throated finch (Poephila cincta), double-barred finch (Poephila bichenovi), red-browed finch (Neochmia temporalis), masked finch (Poephila personata), and blue-faced parrot finch (Erythrura trichroa). The birds ranged from 7 months to 3 years and 8 months of age, and most were acquired from an outside source.

Gross Pathology: The finches presented emaciated with prominent keels and missing feathers on the head and neck. Multiple birds had enlarged gizzards and undigested millet seed throughout the intestines. Gastrointestinal squash preps occasionally revealed mixed bacteria. There were no other significant gross findings.

Histopathologic Description: Ventriculus: The koilin is multifocally disrupted by numerous nematode parasites, parasite eggs, bacterial colonies and mixed inflammatory cells (heterophils and lymphocytes) with associated necrosis. There are multiple cross and longitudinal sections of nematode parasites, the majority of which are sexually mature, gravid females with larvated eggs. The parasites are 200 um in diameter and have a 7-10 um thick, smooth cuticle with 8 um thick transverse striations, polymyarian-coelomyarian musculature, and indistinct lateral cords. The intestine is lined by few, multinucleate enterocytes with a distinct brush border. Eggs are ovoid, non-operculate, and measure an average of 23 x 35 um with a 2-3 um thick refractile shell. Eggs contain either an ovoid embryo or a folded larva. There are multifocal cystic spaces within the koilin (parasite tracts), some of which contain cellular debris, coccoid bacteria and heterophils. There is also multifocal mild lymphoplasmacytic and heterophilic inflammation that expands the lamina propria. Some sections also include proventriculus, with multifocal mild interstitial lymphoplasmacytic inflammation and, variably, clusters of elongate (5 x 20-90 um) eosinophilic yeast within glands or along the mucosal surface at the ventriculus-proventriculus junction (Macrorhabdus ornithogaster). Also, within the proventricular glands of some sections, there are many 3-6 um amphophilic protozoal organisms along the apical surface of mucosal epithelial cells (Cryptosporidium sp.). Few sections contain normal small intestine.

Contributor’s Morphologic Diagnosis: 1. Ventriculus, ventriculitis, heterophilic and lymphocytic, multifocal, severe with koilin disruption and intralesional nematodes, bacteria and yeasts (some sections).
2. Proventriculus, proventriculitis, lymphocytic, multifocal, mild with intralesional yeasts (some sections) and apical protozoa (some sections).

Contributor’s Comment: Ventricular nematodiasis in finches has been reported to be caused by various genera of parasites, including Acuaria, Dispharynx, Tetrameres, Contracecum and Echinura. The morphologic characteristics of these worms in tissue sections and the species affected were consistent with two nematode species: Acuaria skrjabini and Dispharynx nasuta. Examination of whole fixed parasites ultimately confirmed these nematodes to be most consistent with Acuaria skrjabini, a spiruroid.
Acuaria spp. have been reported in cases of finches with a history of diarrhea, lethargy and inappetence.(6) Spiruroid parasites are active parasites of many finch species, especially the ground dwelling species of finch like long-tailed finches, and can be a significant source of mortality.(6) In addition to finches, A. skrjabini has been reported to infect and cause severe disease in sparrows from multiple regions of the world.(8) Fatal A. skrjabini infections with severe ventricular lesions have been reported in avairy finches in Australia and New Zealand.(8) In one population of avairy finches infected with A. skrjabini, there was a reported 66% mortality rate with severe gizzard lesions.(3) Other findings associated with Acuaria spp. include no gross lesions, embryonated eggs in intestinal smears, poor body condition, whole seeds in the intestinal tract, tapeworms in the intestine, and degeneration of the koilin with foci of bacteria.(3,4,8) In this population of finches, small nematodes rarely could be seen at necropsy beneath the koilin layer of the ventriculus.

Acuaria spp. have a two-host life cycle that requires an arthropod intermediate host, which may include beetles, sandhoppers and grasshoppers.(6) Treatment with oral levamisole or fenbendazole has been reported to reduce morbidity and mortality in affected birds,(6) providing supportive evidence that the disruption caused by these spiruroid gizzard worms is the primary cause of this disease syndrome.

Speciation of Acuaria nematodes is difficult, although there are some features to distinguish A. skrjabini. Adult A. skrjabini parasites have seven pairs of postanal papillae in the male, and male cordons reach to and usually beyond the excretory pore.(5) The caudal alae of the male are wider anteriorly.(5) Females are characterized by thick shelled eggs approximately 40-43 x 23-24 µm in the common trunk of uterus.(5) There are also distinguishing features between male and female worms; for example, female worms are about four times as long as the males, and transverse striations at mid body are at about 4 µm intervals in the male while they are at 8 µm intervals in the female.(5)

Yeast forms consistent with Macrorhabdus ornithogaster were also found in the proventriculus of five of the affected birds in this population. M. ornithogaster is a Gram positive, ascomycetous yeast which was originally classified as a bacterium. This organism has been reported in a variety of avian species, including finches, budgerigars and cockatiels. The yeasts are often found at the isthmus between the ventriculus and proventriculus, along the mucosal surface or within proventricular glands. Organisms isolated from chickens are infectious to young chicks, causing reduced weight gain and heterophilic inflammation of the isthmus of the proventriculus.(2)

Cryptosporidia are apicomplexan parasites that infect a wide range of hosts. Cryptosporidia occupy a unique intracellular but extracytoplasmic location on the apical surface of mucosal epithelial cells. Avian cryptosporidiosis can affect the epithelia of the respiratory tract, genitourinary tract, gastrointestinal tract or bursa of Fabricius. In birds, cryptosporidiosis has been reported in chickens, turkeys, quail, pheasants, peafowl, junglefowl, ducks, geese, parrots, finches, lovebirds, and budgerigars. Cryptosporidium meleagridis and C. baileyi are the species that infect turkeys and chickens, respectively. Two novel species, infecting black ducks and finches, have also been described. (7)

AFIP Diagnosis: 1. Ventriculus: Ventriculitis, heterophilic and lymphocytic, multifocal, severe, with intramucosal spirurid eggs, larvae, and adults and numerous yeasts, etiology consistent with Acuaria species and Macrorhabdus ornithogaster, respectively.
2. Proventriculus: Proventriculitis, lymphocytic, multifocal, mild to moderate, with yeasts and many surface epithelial-associated protozoa, etiology consistent with Macrorhabdus ornithogaster and Cryptosporidium species, respectively.

Conference Comment: This interesting case features several infections occurring simultaneously, presenting conference participants with a formidable descriptive challenge. The contributor has masterfully summarized the etiologies involved above. There is substantial slide variation, and not all conference slides include a section of proventriculus.

During the conference, attendees first discussed the differential diagnosis for proventricular and ventricular nematodiasis, and like the contributor, narrowed the list to Acuaria sp. and Synhimatus (Dispharynx) sp. based on the histomorphology of the nematodes and favored Acuaria sp. because of the location. An example of the Synhimatus (Dispharynx) sp. in the proventriculus of a Northern bobwhite quail was reviewed in WSC 2008-2009, Conference 18, case II.
This case was reviewed in consultation with Dr. Christopher Gardiner, Consulting Parasitologist for the AFIP’s Department of Veterinary Pathology, who emphasized that the presence of many small, thick-shelled, embryonated eggs is the most obvious indicator of a spirurid infection. However, the absence of characteristic spirurid eggs is not always sufficient to exclude a spirurid infection, because several spirurids (e.g. Draschia and Thalazia spp.) produce embryos lacking shells. Although there is substantial morphological variety among the spirurids, other features typically observed in members of the group include coelomarian musculature, large intestines lined by uninucleate cuboidal to columnar cells that often have a brush border, large lateral chords that are often vacuolated, and eosinophilic fluid within the pseudocoelom.(1)

Particularly intriguing is the fact that both in this case and in reports in the literature(3,6,8) infection with *Acuaria skrjabini* manifests as an outbreak, despite its requirement for an intermediate host. In one aviary outbreak, a ceramic heat lamp attracted an infestation of cockroaches and earwigs, which were implicated as possible intermediate hosts. The authors noted that while finches of the Estrildidae family normally feed primarily on grass seeds, they transition to a greater dietary intake of insects during the breeding season, which may account for the nearly simultaneous onset of disease in a number of birds. Death of affected birds was attributed to starvation due to maldigestion caused by ventricular dysfunction.(3)

The contributor alluded to the original misclassification of *M. ornithogaster* as a bacterium (based on Gram staining characteristics), giving rise to the misnomer “megabacterium,” by which it was formerly known. *M. ornithogaster* survives only in a microaerophilic environment in a narrow pH range of 3-4, consistent with its peculiar environmental niche at the isthmus of the proventriculus and ventriculus of birds.(2) Although many birds appear asymptomatically infected, the yeast has been implicated in three specific avian disease syndromes: 1) chronic wasting disease in budgerigars, canaries, and finches; 2) acute hemorrhagic gastritis in budgerigars and parrotlets; and 3) reduced feed conversion in experimentally-infected chickens.(2) The clinical significance of the organism in the present case is unclear, but most conference participants interpreted it as an incidental finding, and certainly less important than the spirurids discussed above. By contrast, because they are so numerous and associated with areas of proventricular inflammation, the cryptosporidia in this case are considered clinically significant.

**Contributor:** Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

http://www.hopkinsmedicine.org/mcp/

**References:**

History: A palpable intestinal mass was found on routine examination. Exploratory laparatomy and intestinal resection and anastomosis were performed. The mass was fixed in 10% formalin and submitted for histologic examination.

Gross Pathology: Examination of the fixed tissue revealed large, cystic structures expanding the intestinal wall. The cysts were filled with gelatinous, clear material (mucus).

Histopathologic Description: In sections of jejunum examined, a sparsely cellular mass infiltrates the base of the mucosa, is present in the lamina muscularis, and forms expansile mucus-filled cysts. They diffusely distort the lamina muscularis and submucosa. In some sections, aggregates of mineralized debris are seen in the center of the mucus-filled cysts. Neoplastic cells are columnar to cuboidal or flattened, form small nests or line cyst walls, and are supported by fine to moderately abundant fibrous stroma. Cell layers 1-6 cells thick surround lakes of mucus that at times contain small clusters of cells. Cytoplasm is eosinophilic and faintly granular. Nuclei are round and contain reticular chromatin and one to three distinct nucleoli. Frequently, cells are rounded with abundant foamy amphophilic cytoplasm that peripherally lines the nucleus (signet ring cells). Mitoses (sometimes bizarre) are rare. Neoplastic cells are present in all the terminal sections of the resected segment of intestine examined. In some sections, the surface epithelium is eroded, hemorrhagic and contains rod-shaped bacteria mixed with fibrin. The lamina propria is defined by moderate numbers of neutrophils, few lymphocytes and plasma cells, rare eosinophils and mildly congested blood vessels. Immunohistochemical staining for cytokeratin positively stains enterocytes and neoplastic cells.

Contributor’s Morphologic Diagnosis: 1. Intestine, jejunum: Mucinous adenocarcinoma.
2. Intestine, jejunum: Moderate, sub-acute, multifocal, fibrinosuppurative, erosive enteritis with bacterial rods.

Contributor’s Comment: Intestinal neoplasms in the dog are rare, with primary intestinal neoplasms being more common than metastatic disease.(2,4,6) Primary intestinal neoplasms may arise from the following tissues: epithelium, neuroendocrine cells, mesenchyme (vascular, connective tissue, adipose, or peripheral nerve sheath tumors), lymphoid tissues, extramedullary plasma cells, mast cells, smooth muscle, or serosa.(4) Colonic masses are more frequently encountered, with papillomatous lesions making up the majority of colon tumors.(6) Most are reported to be scirrhous and mucus producing.(2) Other gross lesions can appear as mural thickening, areas of ulceration, white and fibrous tissue, or serosal plaque-like masses.

Transmural invasion is a differentiating feature between adenomatous hyperplasia and neoplasia.(2) Histologic patterns reported include acinar, papillary, solid, carcinoid, and mucinous (including signet-ring cell carcinoma).(6) Signet-ring cells are cells filled with copious mucin that peripherally lines the nucleus. No prognostic behavior has been attributed to the difference in histological pattern.

Intestinal adenocarcinomas metastasize widely via lymphatics, and, at times, via direct serosal implantation.(2) Serosal implantation can cause lymphatic blockage, leading to ascites.(2) Rare manifestations of metastasis include cutaneous masses and pseudomyxoma peritonei.(1,5) Cutaneous lesions in a dog with duodenal adenocarcinoma and masses in numerous organs consisted of undifferentiated, non-cohesive islands of round to polygonal cells.(5) In this case, definitive diagnosis of the undifferentiated mass was supported by identification of a primary intestinal lesion and positive staining of metastases by pancytokeratin, periodic acid Schiff, and Alcian blue. A single case of pseudomyxoma peritonei describing mucin accumulation in the peritoneum and peritoneal cavity was reported in 2003.(1) Mucin was trapped in fibrous reticulin mesh in tissue from the peritoneum and mesenteric fat and in fibrous septa in tissue from the diaphragm. To date, no pathologic basis for mucin accumulation has been identified in this patient or in humans affected by a similar condition.

AFIP Diagnosis: Small intestine: Adenocarcinoma, mucinous type.

Conference Comment: The contributor provides a fairly straightforward case of mucinous adenocarcinoma in the jejunum of a dog, pairing it with a succinct synopsis of the entity. The conference moderator cautioned participants to carefully consider adenocarcinoma, which in dogs is more common than intestinal adenoma, whenever glands are present in the submucosa, tunica muscularis or serosa, even in cases with a predominance of histologically bland neoplastic cells; tumor cells in intestinal adenocarcinoma can be well-differentiated, a finding that may vary regionally within a given malignant neoplasm. This case is typical in that intestinal neoplasms of epithelial origin are more commonly found in males than females, whereas the opposite is true for nonepithelial intestinal tumors. Of note, adenocarcinomas in dogs are more common in the large intestine than in the small intestine.(3)
The following summary of the World Health Organization classification of intestinal adenocarcinoma is provided with the caveats that a given tumor often exhibits more than one growth pattern, and as mentioned by the contributor, growth pattern is not correlated with prognosis:(3)

<table>
<thead>
<tr>
<th>Classification of Intestinal Adenocarcinoma in Domestic Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td>Acinar (tubular)</td>
</tr>
<tr>
<td>Papillary (polypoid, cribriform)</td>
</tr>
<tr>
<td>Mucinous (colloid, mucoid)</td>
</tr>
<tr>
<td>Signet ring cell (goblet cell, intracellular mucinous)</td>
</tr>
<tr>
<td>Undifferentiated</td>
</tr>
<tr>
<td>Adenosquamous (adenoacanthoma, adenocarcinoma with squamous differentiation)</td>
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</table>

Contributor: Veterinary Diagnostic Center, University of Nebraska, 1900 North 42nd Street, Lincoln, NE 68583
http://nvdlsl.unl.edu/

References:

CASE III: 12303-08 (AFIP 3134626).

Signalment: 5-year-old, female, spayed, domestic shorthair cat (*Felis catus*).

History: The cat had neurologic signs that ended in torticollis and vocalization.

Gross Pathology: There were no gross lesions in the viscera, brain, or spinal cord.


Histopathologic Description: A diffuse and severe influx of neutrophils was present throughout the brainstem. Only slight lymphocytic perivascular cuffing with a few neutrophils was in the adjacent cerebellar white matter and focally in the posterior cerebral white matter and midbrain. Mostly neutrophils with some lymphocytes and histiocytes surrounded the vessels and infiltrated and thickened their walls with mostly neutrophils within the brain parenchyma. Occasionally, the cytoplasm of degenerating neurons was filled with neutrophils (neurophagia). The
trigeminal ganglia (not on slide) were collected from the refrigerated body about three days later, and one also had mild lymphocytic inflammation, a few neutrophils, and multiple colonies of short gram-positive bacilli, compatible with *Listeria* ("cold enrichment"). Inflammation was absent in the meninges except for occasional perivascular inflammation in the subarachnoid spaces of the brainstem.

**Contributor’s Morphologic Diagnosis:** Diffuse, suppurative brainstem encephalitis.

**Contributor’s Comment:** This is neurologic listeriosis as would be expected in a ruminant. In ruminants, infection is generally associated with feeding silage. The bacteria can live if the silage pH is above 5.5. In Arkansas, we see sporadic cases in cattle and goats on pasture and eating round bales of hay that may be spoiling on the ground side. Infection gains entry to a mouth lesion and the bacteria travel up axons of the trigeminal nerve to the trigeminal ganglion and brainstem. The characteristic lesion is brainstem microabscesses or glial nodules that eventually fill with neutrophils, and lymphocytic perivascular cuffing and local brain parenchymal edema and rarefaction. Mild meningitis of the cerebellum and anterior spinal cord is common. Encephalitic cases are rare in dogs(2) and no report was found of neurologic listeriosis in a cat and few septicemic cases.(3) Listeriosis typically has one of three separate entities: encephalitis, septicemia, or metritis-abortion.(3) The lesions in this cat are much more suppurrative than we usually see in ruminants.

**AFIP Diagnosis:** Brainstem and cerebellum: Encephalitis, suppurative, multifocal to coalescing, marked.

**Conference Comment:** In addition to the lesions described by the contributor, conference participants noted reactive astrocytosis and rare foci of neutrophilic inflammation within the cerebellar white matter on some slides. Several conference participants considered feline infectious peritonitis (FIP) in the differential diagnosis. However, several histologic findings argue against a diagnosis of FIP: 1) inflammation elicited in FIP is classically pyogranulomatous, whereas the inflammation in this case is predominantly neutrophilic; 2) neutrophils in the pyogranulomatous inflammation of FIP are classically nondegenerate, whereas the neutrophils in this case are mostly degenerate; and 3) inflammation in FIP is generally centered on blood vessels, ventricles, and choroid plexus, whereas in this case, foci of inflammation are scattered randomly throughout the neuropil of the brainstem. The tissue Gram stain demonstrates numerous gram-positive short bacilli, and in congruence with the reported bacterial culture results, solidifies the diagnosis of encephalitic listeriosis.

In humans and animals, *L. monocytogenes* is credited with producing three distinct syndromes, each of which is thought to develop by a unique pathogenesis. The first, as demonstrated by this case, is encephalitis, which occurs almost exclusively in ruminants and is associated with feeding incompletely fermented silage where the bacterium readily multiplies, as described by the contributor. Interestingly, although *L. monocytogenes* has been shown experimentally to breach the blood-brain barrier, the distribution of lesions in naturally-occurring cases of listerial encephalitis suggests direct invasion through the oral mucosa, ascension through the trigeminal nerves, and centripetal travel via axons to the brain, rather than hematogenous infection. Preferentially affected are the medulla and pons, with less severe infection in the thalamus and cervical spinal cord. Classically, clinical signs consist of depression, head-pressing, and circling, often with unilateral paralysis of the seventh cranial nerve and resultant facial drooping and/or unilateral purulent endophthalmitis, which may be confused with malignant catarrhal fever.

(2) The second syndrome is abortion, which is thought to result from hematogenous infection of the pregnant uterus following an asymptomatic bacteremic phase. In cattle and sheep, abortions occur in the third trimester of pregnancy, often without clinical illness in the dam, followed typically by retention of the placenta. Infection in the early part of the third trimester results in expulsion of an autolyzed fetus. Late third trimester infections produce the third listeriosis syndrome, septicemia, characterized by miliary yellow foci of necrosis and bacteria that are grossly evident in the liver, and microscopically also found in the lung, heart, kidney, spleen, and brain. Both the cotyledonary and intercotyledonary regions of the placenta are affected by a robust necrotizing placenitis. Interestingly, it is uncommon for the encephalitis and abortion syndromes to occur together in a herd or flock.(4)

In addition to the remarkable ability of this ubiquitous saprophyte to survive and grow in the environment, *L. monocytogenes* persists indefinitely in macrophages, thereby escaping the humoral immune system. The bacterium induces phagocytosis via a cell surface protein called internalin; inside the cell it escapes phagosome-mediated killing via such virulence factors as listeriolysin O and phospholipases. Then, in a most impressive display of stealth, *L. monocytogenes* manipulates host cell contractile actin to facilitate movement from cell to cell without exposure to antibodies.(1,2)
**Contributor:** Arkansas Livestock and Poultry Commission, #1 Natural Resources Drive, Little Rock, AR 72205

**References:**

**CASE IV: 07-21444 (AFIP 3152276).**

**Signalment:** 10-year-old male Labrador retriever dog (*Canis familiaris*).

**History:** This dog had a clinical history of "CNS signs." The dog was a stray, and because it was acting "odd" according to the property owner, the dog was submitted to the laboratory for rabies testing. The brain was removed and the rest of the body was not examined.

**Gross Pathology:** The cerebrum was distorted by an approximately 2 x 2.5 cm spherical, pale yellow-tan soft friable mass. The mass was not encapsulated and was not connected to the meninges.

**Laboratory Results:** Direct FA for rabies virus antigen was negative. An aerobic culture of the brain was positive for *Pasteurella* sp.

**Histopathologic Description:** The meninges and adjacent neuropil have been infiltrated by numerous variable-sized nests of plump, round neoplastic cells. Individual nests are separated by a delicate fibrovascular stroma. The neoplastic cells have finely granular eosinophilic cytoplasm, a central or eccentric, small, finely stippled nucleus with one or two nucleoli. Mitotic figures are rare (not visible on all slides) and in some slides there are microfocal lymphocytic perivascular cuffs within the tumor or the adjacent unaffected neuropil.

**Contributor’s Morphologic Diagnosis:** Intracerebral granular cell tumor.

**Contributor’s Comment:** In rats, granular cell tumors are the most common intracranial tumor.(5) In horses, this type of tumor has only been reported in the lungs, where it may be an incidental finding.(4) In dogs, granular cell tumors are most commonly located in the oral cavity.(2) Other, less frequently reported sites in dogs include the heart, skin and brain.(1) Other species with reported granular cell tumors include cats(6) and ferrets.(5) Granular cell tumors arising in the peripheral soft tissues of non-human animals are generally believed to originate from Schwann cells.(1,4) The tumor cells usually contain diastase-resistant PAS-positive cytoplasmic granules.(1,3) A definitive origin for granular cell tumors in the CNS of non-human animals has not been determined.

In humans, these tumors are histogenetically heterogeneous and occur both within the nervous system and in extra-neural locations.(3) The tumors in the CNS are believed to arise from astrocytes or pituicytes and tumors in extra-neural sites are believed to be of peripheral nerve origin (usually Schwann cells).(6)

In previously reported cases of intracranial granular cell tumors, the clinical signs have included blindness (when the tumor entraps the optic nerves), seizures, ataxia, weakness, nystagmus, opisthotonus, and proprioceptive deficits. (1,3,5,6) Unfortunately, the history in this particular case was limited to "CNS signs" and "acting odd."

In this case, small perivascular cuffs of lymphocytes and less frequently neutrophils were present in the non-involved cerebrum and hippocampus (not included in most slides). In the cerebellum and hippocampus the endothelial cells lining most blood vessels were plump and hypertrophic (reactive endothelial cells). Perivascular cuffing associated with this type of tumor has been reported previously(1), and in this case we assume that the lymphocytic perivascular cuffing is associated with the tumor while the neutrophils and reactive endothelium may be in response to the presence of a *Pasteurella* sp., believed to be an acute infection.
AFIP Diagnosis: Cerebrum: Granular cell tumor.

Conference Comment: This case was reviewed in consultation with the AFIP Department of Neuropathology. It is perplexing that while the gross description indicates that the neoplasm was not attached to the meninges, microscopic evaluation reveals that the neoplasm, indeed, expands the meninges, often obliterating Virchow-Robin space, and infiltrates the adjacent neuropil. As indicated by the contributor’s comments, the diverse list of examples of granular cell tumors in various species suggests that the granular cell phenotype may develop in tumors that have different cells of origin. While the histogenesis of granular cell tumors from various anatomic locations in several animal species is known, the cell of origin of the cerebral granular cell tumor remains enigmatic.

Conference participants briefly discussed oncocytoma and laryngeal rhabdomyoma in the dog; these neoplasms share histological similarities with granular cell tumors. Electron microscopy indicates that the prominent cytoplasmic granules in granular cell tumors are lysosomes and autophagosomes.(1,3) In oncocytomas and laryngeal rhabdomyoma, the cytoplasm is packed with mitochondria, accounting for the granules seen on H&E.(7)

Contributor: Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Veterinary Science Department North Campus Drive, Brookings, SD 57007
http://vetsci.sdstate.edu

References: