

**The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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**CONFERENCE 5
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CASE I – no slide label (AFIP 2596193)

Signalment: 5-month-old emu, from the order Struthioniformes, scientific name-
Dromiceius novaehollandiae.

History: This emu was found dead with no previous signs of illness. Housed in a pen of 150 others. No other pen mates noticed to be sick. These birds are being raised for meat and oil. This carcass was submitted frozen.

Gross Pathology: Bird is in poor body condition. The cranial and caudal thoracic and abdominal air sacs contain multifocal to coalescing, yellow to white, raised plaques, some with dark-green blue centres and visible mycelia. Similar discrete, white to pale yellow foci are scattered throughout the lung, and fill primary bronchi.

Laboratory Results: This case was not cultured. *Aspergillus fumigatus* had been recovered from previous cases of emus with similar lesions.

Contributor's Morphologic Diagnosis: Severe chronic multifocal mycotic pneumonia and airsacculitis - *Aspergillus fumigatus*

Contributor's Comment: In Ontario, over the past two winters, we have seen several cases of mycotic pneumonia and airsacculitis with *Aspergillus fumigatus* being recovered by fungal culture. The clinical signs can be very mild with birds showing slight mouth breathing after running and, in some cases, birds were noted only to be unsteady on their feet. Because of these subtle clinical signs, the early identification of sick birds is very challenging and, on most farms, until a bird dies and is necropsied, the producer is unaware of any problems.

The condition is seen more commonly in the fall and winter when the birds are confined indoors with inadequate ventilation systems. Greenhouse set-ups are very much in vogue in this area and the ventilation is extremely poor. Practitioners from our area have described several cases of mycotic pneumonia and airsacculitis in emus being raised in these set-ups.

AFIP Diagnosis: Airsac: Airsacculitis, heterophilic and granulomatous, diffuse, severe, with numerous hyphae and conidiophores, etiology consistent with *Aspergillus fumigatus*, emu (*Dromiceius novaehollandiae*), avian.

Conference Comment: *Aspergillus fumigatus* is a ubiquitous, saprophytic, opportunistic pathogen that commonly causes infection in many avian species. Typical *Aspergillus sp.* hyphae are septate, parallel-walled, have a uniform width of 3-6 µm, with acute angle dichotomous and progressive branching. The conidiophores of *A. fumigatus* are 5-8 µm in diameter, up to 300 µm in length, and gradually enlarge into a flask-shaped vesicle that is 20-30 µm in diameter and bear a single row of phialides. The phialides produce radiating chains of conidia that are 2-3 µm in diameter. In adult ratites, *A. fumigatus* respiratory infection is acquired via inhalation of dust from dry feeds and soil that are infected with conidia. In young ratites, outbreaks of aspergillosis are commonly associated with immunosuppression, stress, inadequate hygiene, and antibiotic administration. Suggested control measures include restricting access to organic debris, stress reduction, minimizing antibiotic usage, and ensuring adequate ventilation. In ratites, aspergillosis can result in carcass condemnation. Aspergillosis has been observed in horses, cattle, dogs, cats, reptiles, and marine mammals.

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CASE II - AR-01-532 WFUSM (AFIP 2839457)

Signalment: 14-year-old, male, pigeon (*Columba livia*), White Carneau

History: This bird was examined for rapid respiration, at which time it was noted that the abdomen was distended and firm on palpation. The bird was euthanized due to advanced age and poor prognosis.

Gross Pathology: An adult white male pigeon, in fair body condition, without postmortem changes or external lesions was examined. Two, tan, variably firm ovoid masses, one multinodular, 5x3x3cm, and the other, smooth, 3x2x2cm, were found in the dorsal coelomic cavity on the left and right sides of midline, respectively. Multifocal hemorrhage and necrosis were noted on cut surfaces.

Laboratory Results: None.

Contributor's Morphologic Diagnoses: Seminoma, bilateral, testicles

Contributor's Comment: Seminomas are common canine testicular tumors but are rarely reported in horses, cattle, sheep and goats, and birds. They occur in older animals, frequently in cryptorchid testes, arising from cells of the spermatogenic series, and are occasionally malignant and locally invasive (Jubb *et al*, 1993). Seminomas have been reported in several avian species, including cockerels, budgerigars, an Amazon green parrot, a Jardine Warbler, a duck, a pigeon, a black swan and a collared turtle dove (Hemboldt *et al*, 1991; Turk *et al* 1981; Ganorkar *et al* 1998; Dillehay *et al*, 1990). Clinical presentation includes lethargy, anorexia, increased respiration, diarrhea, and abdominal distention (Turk *et al*, 1981), with gross findings similar to those in this case.

Microscopically, both tumors were encapsulated and composed variably of sheets, packets and tubule-like structures resting on basal laminae, separated by variably thick fibrovascular stroma, interspersed with foci of hemorrhage and necrosis. The neoplastic cells had pale vesicular, generally round 6-12 um in diameter nuclei with finely stippled chromatin, often with central to paracentral 1-2 um in diameter nucleoli, and moderate amounts of vacuolated to finely granular pale eosinophilic cytoplasm. Cell borders were generally indistinct. Mitoses averaged 2 per 40X field, and small perivascular accumulations of lymphocytes were scattered within the peripheral stroma.

Electron microscopy confirmed the presence of a basal lamina supporting rows of neoplastic cells without intercellular junctional complexes, with few cytoplasmic organelles, abundant cytoplasmic vacuoles, and round nuclei with single or indiscernible nucleoli. Sertoli cells would be expected to have junctional complexes and oval nuclei, often with characteristic primary and accessory nucleoli. Leydig cells typically have abundant cytoplasmic droplets characteristic of steroid secreting cells.

The incidence of avian seminomas has been documented only in budgerigars, in which species it is reported as 3-9.25% of all tumors examined (Turk *et al*, 1981). Although most are benign, (Dillehay *et al*, 1990) the location and large size of the tumors at the time of diagnosis discourages surgical excision. Diagnosis is based on clinical findings, and is usually confirmed at necropsy (Turk *et al*, 1981).

AFIP Diagnoses: Testicle: Seminoma, White Carneau pigeon (*Columba livia*), avian.

Conference Comment: The contributor has provided a concise summary of avian seminomas. By immunohistochemistry, human seminomas are positive for placental-like alkaline phosphatase (PLAP), vimentin, and *c-kit* (CD 117a). In this case of avian

seminoma, neoplastic cells did not stain for PLAP or *c-kit*. These tests may not work in birds.

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CASE III – A433343 (AFIP 2838983)

Signalment: 7 year-old, female, Red eared slider, *Trachemys scripta elegans*, Turtle.

History: The turtle was presented to the Animal Medical Center initially for a 2-3 week period of anorexia, lethargy and reduced egg laying. The turtle had laid eggs annually but had produced none the year of presentation. Physical examination determined open mouth breathing subsequent to a distended coelom and edema of the proximal subcutaneous tissues of all four limbs. Coelomic radiographs revealed three unshelled eggs in the right cavity. Hormone therapy failed to induce oviposition and she presented one month later for the onset of a painful abdomen. Endoscopy under light anesthesia did not reveal abnormalities. An exploratory laparotomy via plastron osteotomy was performed, and a large, 450 gram, encapsulated, lobulated, firm mass was removed from the mid-coelom close to the cranial aspect of the left uterine horn. The turtle died intra-operatively.

Gross Pathology: The 450 gram, 10x12x5 cm mid-coelomic space occupying mass was submitted for examination. It was firm and variegated tan, white and red. Scattered over the capsular surface and on cut section were numerous tan and white pale gritty foci measuring 0.1–0.5 cm in diameter. The bulk of the mass was fibrous with scattered cystic spaces containing mucoid material.

Laboratory Results: On presentation, hyperglycemia, hypercalcemia, uremia, hypoproteinemia and elevated AST 227 (5-55) and CK 472 (64-440) values were determined. Additionally, anemia, neutropenia, lymphopenia, eosinopenia and basophilia were recorded.

Contributor's Morphologic Diagnosis: Malignant ovarian teratoma with intratumoral multifocal random acute epithelial cell necrosis, fibrin exudation and acute hemorrhage

Contributor's Comment: Teratomas are neoplasms containing elements originating from more than one germ cell layer. These elements are either seen in associations that resemble normal organs or are intermingled haphazardly. Teratomas may be classified as benign (mature) or malignant (immature) depending on the degree of anaplasia or the presence of undifferentiated elements resembling those of the embryo. Teratocarcinomas are undifferentiated malignant neoplasms, which contain histologic features of teratoma and embryonal carcinoma, choriocarcinoma or both. Teratomas most commonly arise from gonadal tissue and have been reported in the ovary of horses, pigs, cattle, dogs, cats, rodents, humans, monkeys, domestic fowl, woodchucks, squirrels, lizards, and a guppy. Extragonadal sites of teratoma formation, including intracranial, peritoneal, and retroperitoneal sites, have been documented rarely in dogs and birds.

There are few published reports of gonadal neoplasms in reptiles and very few in chelonians. Scant literature has characterized germ cell neoplasms in fish, lizards, and in turtles. A dysgerminoma has been reported in a snapping turtle. This is the first report of an ovarian teratoma in a turtle.

This case represents the first report of a histologically malignant gonadal teratoma in a chelonian. Although most gonadal teratomas from animals are benign, histologically malignant teratomas, have been reported. Malignant teratomas contain less well-differentiated embryonal elements in addition to mature structures, increased cellular atypia and regional overgrowth of a single tissue type. Additionally, they often show multicentric growth secondary to direct implantation or distant metastases. In this turtle, the teratoma was determined to be malignant because of the foci of anaplastic epithelial glandular cells. These foci were often associated closely with cartilaginous tissue. Additionally, these epithelial areas were also concurrently necrotic with variable amounts of associated fibrin exudation and fresh hemorrhage. Although non-coelomic metastases could not be ruled out, metastatic or implanted masses were not noted within the accessible coelomic cavity at the time of the surgical resection and laparotomy.

The theories on histogenesis of teratomas are fascinating but controversial and may vary depending on whether they arise in a gonad or an extragonadal site and the sex of the patient. In humans, ovarian teratomas have been suggested to be parthenogenic, and develop from a single germ cell, which has completed its first meiotic division but not its second. In the case of extragonadal tumors, premeiotic germ cells with a diploid chromosome set that have not yet undergone the first meiotic division or pluripotent ectopic embryonal or extraembryonic cells are considered the cells of origin. The former occurs following suppression of the second meiotic division or by fusion of the second polar body. The latter may originate from blastomere

segregated during early embryonic development or from displaced yolk sac remnants. The cells in ovarian teratomas are typically XX diploid cells, which are homozygous at chromosomal loci for which the host is heterozygous although examples of those with chromosomal abnormalities also do exist.

Most case reports of teratomas refer to mammals, although a few have been reported in birds and reptiles. In mammals, teratomas are typically single benign masses that replace ovarian tissue, but rare reports of extragonadal teratomas also exist. No normal ovarian tissue was examined from this case, but exploratory laparotomy indicated that the mass was present in the correct anatomic location (i.e. left ovary) and mesovarium surrounded the mass. Although ovarian neoplasia is particularly rare in chelonians, dysgerminomas and teratomas should be considered as differential diagnoses for gonadal neoplasia in turtles.

This is an interesting and unique case because it represents one of the few reports of gonadal neoplasia in the turtle and the first report of a malignant teratoma in any turtle species. Most of the published case reports have occurred in mammals, although the more exotic species affected include birds, a fish and a lizard. These tumors typically occur in gonadal tissue, most consistently in the ovary. Ovarian tissue was not detected in any of the 24 examined sections but it is the presumed site of origin of this mass lesion based on consultation with the surgeon. Although metastatic disease was not detected at the time of abdominal surgery, the teratoma shows histologic characteristics of malignancy and thus transabdominal or hematogenous spread would likely have occurred. Unlike in canine malignant teratomas where the anaplastic population is composed of the mesenchymal component it was the epithelial component that was malignant in this case.

AFIP Diagnosis: Ovary (per contributor): Mixed germ cell tumor, teratoma (90%) mature and immature, and yolk sac tumor (10%), red eared slider turtle (*Trachemys scripta elegans*), chelonian.

Conference Comment: The contributor has provided us with an interesting and thought provoking case and an excellent review of teratoma. This case sparked the most discussion both during and after this conference. Conference participants essentially agreed with the contributor's diagnosis of malignant teratoma for the reasons provided above. However, some conference participants identified a few small foci of primitive neoplastic cells interpreted as yolk sac tumor. These neoplastic cells are arranged in a loose meshwork or reticular pattern; are cuboidal to polygonal; and have variably distinct cell borders, a small amount of pale to clear cytoplasm, irregularly round to oval nuclei with stippled chromatin, and usually two small magenta nucleoli. Mitotic figures are evident in this population as well as individual cell necrosis. By immunohistochemistry, these primitive neoplastic cells are negative for cytokeratin and weakly positive for alpha-fetoprotein; consistent with yolk sac origin.

These findings prompted considerable discussion on the intricacies of teratoma nomenclature and the proper name for this particular lesion, and eventually led us to a consultation with Dr. Isabell Sesterhann of the Department of Genitourinary Pathology, Armed Forces Institute of Pathology. Dr. Sesterhann agreed with the interpretation of a yolk sac component and, after additional discussion, we arrived at the above diagnosis.

Mixed germ cell tumors are, as the name implies, combinations of more than one histologic type of germ cell - in this case, teratoma and yolk sac tumor. In human medicine, these different histological components are listed in descending order by percentage. In this specimen, the teratoma contains immature tissues and mature tissues representing all three embryonic layers. A single focus of immature tissue justifies the diagnosis of immature teratoma and indicates malignancy. Yolk sac tumors are primitive, malignant germ cell tumors with many distinctive microscopic patterns.

We are grateful to Dr. Sesterhann for her assistance with this case.

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CASE IV - 01-318 (AFIP 2834689)

Signalment: 16 year-old, male, Tundra swan

History: There was an approximately 6-month history of decreased activity and loss of appetite. Previous Tundra swans in this exhibit had died of aspergillosis. This swan was tube fed and treated with oral Baytril and Sporanox for several weeks, after which

time he began eating on his own. After several weeks and inconclusive results from laboratory tests, he appeared to recover and was eventually placed back on exhibit. He eventually began showing signs of decreased activity, but died unexpectedly before additional examination was performed.

Gross Pathology: There was a 2x2x3 cm granuloma within the left lung with adhesions to the adjacent pericardium. There was also a large fresh green leaf folded within the caudal part of the mouth that covered the opening to the pharynx. The leaf was stuck to the upper and lower mouth and appeared to have occluded the pharynx adequately to suffocate the bird.

Laboratory Results: *Cladophialophora spp.* was isolated from the pulmonary lesion and was confirmed by Dr. Rinaldi's Fungal Testing Lab, Univ. of Texas, Dept. of Path., San Antonio, Texas, to be *Cladophialophora bantiana*.

Contributor's Morphologic Diagnosis: Granulomatous pneumonia, Phaeohyphomycosis, *Cladophialophora bantiana*

Contributor's Comment: *Cladophialophora bantiana* is a relatively new and uncommonly recognized opportunistic fungal pathogen of humans and animals. Medline currently shows 262 citations of phaeohyphomycosis, including 23 citations of *Cladophialophora bantiana*, which report various aspects of this organism and the disease it produced in primarily immune suppressed people. There is a case report of fatal cerebral infection caused by *Cladophialophora bantiana* in a dog.

The infection results in granulomatous inflammation, which contains pigmented fungal hyphae. The fungal hyphae are reported to be 7-9 um wide, contain septa, and brown to green pigment. *Cladophialophora sp.* can be identified microscopically from cultures based upon long chains of smooth, brown or pigmented ellipsoidal, unbranched conidia arising from septate conidiophores after staining with lactophenol aniline dye. Species identification is typically made at the Fungal-testing Laboratory in San Antonio, Texas. The infection was restricted to a pulmonary granuloma in the Tundra swan, but systemic lesions within brain, liver and kidneys were reported in a dog.

AFIP Diagnosis: Lung: Pneumonia, granulomatous and heterophilic, diffuse, severe, with pigmented hyphae, consistent with phaeohyphomycosis, Tundra swan (*Cygnus columbianus*), avian.

Conference Comment: The phaeohyphomycoses are a heterogeneous group of ubiquitous, saprophytic, opportunistic, dematiaceous fungi that primarily cause subcutaneous and systemic infections, and uncommonly, cerebral and cutaneous lesions in cats and dogs. Phaeohyphomycosis also has been reported in horses, cattle, and birds. Typical phaeohyphomycotic hyphae are septate and branching, with non-parallel and variably pigmented walls, and have a non-uniform width of 2-6 um, with random, vesicular swellings up to 25 um in diameter.

Melanin is the pigment in the hyphae and a known virulence factor in fungi. It may serve to scavenge free radicals and hypochlorite produced by phagocytic cells and/or it

may bind hydrolytic enzymes. Melanin is also believed to function in the formation of the fungal appressorium, a structure that aids in entering the host cells.

Cladophialophora bantiana has many synonyms including *Cladosporium bantianum*, *Xylohypha bantiana*, and *Cladosporium trichoides*. This particular phaeohyphomycotic fungus is neurotropic and is often associated with cerebral lesions. The portal of entry is unknown, but inhalation followed by hematogenous dissemination is suspected. In animals, most cases have been described in dogs and cats. There are reports of disseminated disease as well as reports of only neurologic involvement.

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