



## WEDNESDAY SLIDE CONFERENCE 2015-2016

# Conference 6

2 November 2015

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### CASE I: NCAH-2012B (JPC 4018127).

**Signalment:** 77 month old female raccoon (*Procyon lotor*).

**History:** This individual was used as a negative control in a transmissible spongiform encephalopathy (TSE) transmission study. This animal died 68 months after the experimental cohorts in the study were inoculated with a TSE.

**Gross Pathology:** The uterus was markedly distended, appearing to occupy the majority of the abdominal cavity. The wall of the uterus was uniformly thin, and contained approximately 300mL of a clear, thin liquid (hydrometra). The oviducts were moderately thickened, and the ovaries appeared small. The stomach was moderately distended, containing approximately 50mL of dark black mucoid material and had multifocal hemorrhages. The intestinal tract appeared compressed and did not contain any ingesta. Microscopic lesions were bilateral and confined to the uterus and oviducts.

**Laboratory Results:** None

**Histopathologic Description:** Uterus: Endometrial glands are variably dilated by clear

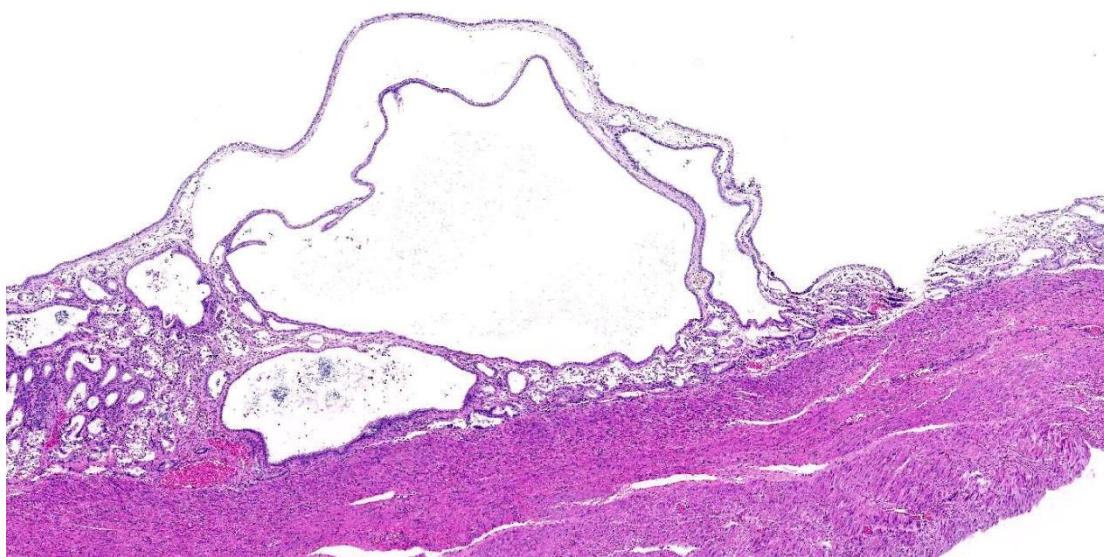


*Uterus, raccoon. The mucosa is studded with multifocal cystic endometrial glands. (HE, 6X)*

space and scant to moderate amounts of eosinophilic fluid and/or viable and degenerate epithelial cells or cystic, up to 4 mm in diameter. Glands are haphazardly arranged and the epithelium lining glands and cysts are short to tall columnar, occasionally with intact cilia, line rare papillary projections, or are occasionally piled up four to five cells thick. Nuclei are round to oval with finely stippled basophilic chromatin and a single lum magenta nucleolus. Small to moderate numbers of viable and degenerate neutrophils are rarely present in the loose connective tissue of the submucosa, which is atrophied. The myometrium is variably thin (section variation) and myometrial blood vessels are dilated by red blood cells (congestion).

**Contributor's Morphologic Diagnosis:** Uterus: Endometrial hyperplasia, cystic, diffuse, chronic, mild to moderate.

**Contributor's Comment:** Endometrial hyperplasia is common in domestic canines, often involves cystic distension of endometrial glands (cystic endometrial hyperplasia, CEH) and may result in bacterial infection (pyometra). A variety of sterile substances have been placed in the uterine lumen of bitches during the luteal (sometimes referred to as secretory or progestational) phase, which resulted in endometrial hyperplasia and remodeling. Another theory suggests a low-grade, subclinical bacterial infection during the luteal phase results in endometrial proliferation. CEH can affect a single or just a few glands, affect glands segmentally, or diffusely affect the entire endometrium.



*Uterus, raccoon: Cystic endometrial glands range up to 2mm in diameter. (HE, 20X)*

Some species develop endometrial hyperplasia as a result of excessive and prolonged estrogenic stimulation. Estrogen contributes to the pathogenesis of endometrial hyperplasia by priming the endometrium, via estrogen receptors, to induce the synthesis of intracellular receptors for progesterone.<sup>11</sup> Progesterone, from ovarian corpora lutea (CL), then induces the proliferation

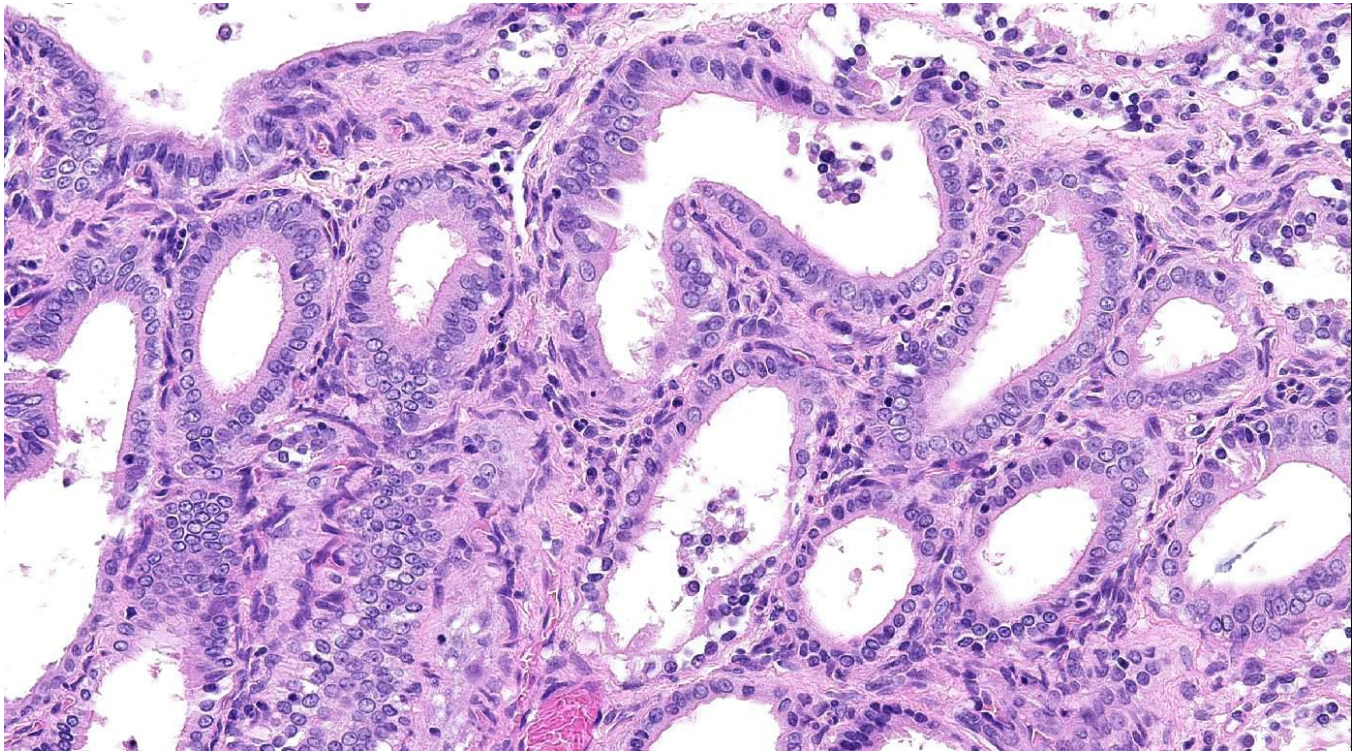
and secretion of the endometrium.<sup>6</sup> In addition to the normal estrous cycle, estrogen sources may be endogenous, as with granulosa cell tumors, or exogenous, as with phytoestrogens.<sup>11</sup> Exogenous sources of progesterone can be found in melengestrol acetate (MGA), megestrol acetate, and medroxyprogesterone.<sup>7</sup>

Chronically hyperplastic endometrial glands lead to the gross accumulation of mucoid fluid. Mucometra and hydrometra are considered by some to be variations of the same condition, their difference lying in hydration of the mucin secreted by the endometrium. In addition to the accumulation of fluid concurrent with endometrial hyperplasia, it may also result from the obstruction of a segment of the uterus, cervix, or vagina. If muco/hydrometra persists, the pressure caused by the accumulating fluid results in the attenuation/atrophy of the endometrium

and thinning of the uterine wall.<sup>11</sup>

In addition to domesticated animals, cystic endometrial hyperplasia has also been described in raccoons,<sup>3,4</sup> Asian and African elephants,<sup>1</sup> African hunting dogs (*Lycaon pictus*),<sup>6</sup> a chinchilla,<sup>2</sup> and multiple zoo

canid species.<sup>7</sup> With the exception of one primiparous individual, all elephants with CEH (15 total) were nulliparous. Asian elephants had a significantly greater risk of developing CEH while the risk for both Asian and African elephants increased with age.<sup>1</sup> The three African hunting dogs were multiparous; two had CLs and two had ovarian granulosa cell tumors.<sup>6</sup> The chinchilla was nulliparous and had a history of



*Mildly dilated gland contain small amounts of sloughed cells, protein, and cell debris. Lining cells are tall, columnar, and lack the characteristic vacuolation of cells under progesterone influence. (HE, 168X)*

blood being found sporadically in its cage.<sup>2</sup> Zoo canids that had been treated with MGA as a contraceptive were significantly more likely to develop CEH than non-treated canids.<sup>7</sup>

Of the four female raccoons used in the study, three developed endometrial hyperplasia; the uterus of the fourth was not examined. None of these raccoons had received any exogenous hormone therapy nor were any ovarian cysts/tumors or uterine, cervical, or vaginal obstructions identified. Finally, all of the raccoons were nulliparous.<sup>4</sup> The likely pathogenesis of cystic endometrial hyperplasia and resultant mucometra in this case may therefore be similar to that of domestic carnivores (i.e. driven by prolonged and/or repeated estrogen priming followed by progesterone stimulation).

#### **JPC Diagnosis:**

1. Uterus: Multiple endometrial cysts.
2. Uterus, muscular tunic: Adenomyosis, multifocal.

**Conference Comment:** Conference participants' histologic description was largely aligned with the contributor's description. Additional features discussed included infiltration of the uterine stroma by low numbers of lymphocytes and macrophages, a scant amount of mineral admixed with the sloughed epithelial cells and few multifocal areas of uterine adenomyosis. Although the lesion was initially interpreted as cystic endometrial hyperplasia by conference participants, the moderator offered an alternative interpretation. Although admittedly within the current literature, cysts within endometrium are almost universally described with reference to cystic endometrial hyperplasia, the moderator noted that CEH is generally more of a proliferative lesion, and in this case the lesion was minimally proliferative and predominantly only cystic. Thus, this lesion may represent an age-related change which occurs in mammals and is unrelated to estrogenic stimulation and development of hyperplastic uterine mucosa. Schlafer and Gifford have accurately stated that describing and interpreting proliferative and cystic lesions within the endometrium is often "difficult, frustrating and

potentially confusing,”<sup>10</sup> which serves to highlight the potential for contention in a case such as this.

There are two patterns of cystic endometrial hyperplasia described in the bitch: generalized CEH and pseudoplacental endometrial hyperplasia (PEH), also referred to as localized endometrial hyperplasia of pseudopregnancy. Both may result in accumulation of secretions within the uterus, but in PEH there will also be cellular debris present from necrosis of the superficial endometrium, which may be confused with pyometra.<sup>9</sup> Additionally, in PEH the proliferation is highly organized and histologically similar to placentation sites seen in pregnancy.<sup>10</sup> In contrast to CEH, noncystic endometrial hyperplasia is generally only recognized microscopically and characterized by irregular proliferation and arrangement of uterine glands. The stroma is generally edematous, the mucosal epithelium hypertrophied, and adenomyosis may be present.<sup>9</sup> Adenomyosis refers to endometrial tissue located within the muscular layers of the uterus. It often appears like normal uterine glands which may be associated with secretory material, or the glandular epithelium may be atrophied leaving only connective tissue and resulting in a cyst like structure in the myometrium.<sup>10</sup>

In the cow CEH is associated with granulosa cell tumors, ovarian follicular cysts as well as exposure to exogenous sources of estrogen.<sup>9, 11</sup> In the mare and camelids it is considered very uncommon and not associated with granulosa cell tumors.<sup>9</sup> CEH has also been observed as a common lesion in miniature pigs, which are often kept as pets and therefore live longer than domestic pigs. In the sow CEH has also been associated with the mycotoxin zearalenone.<sup>5</sup> CEH has also been described in sheep associated with ingestion of estrogen containing plants as well as in goats without association with ingestion of estrogenic plants.<sup>8</sup>

#### **Contributing Institution:**

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www.nadc.ars.usda.gov

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**CASE II:** E274/14 (JPC 4048928).

**Signalment:** Juvenile, female bronze-winged Parrot (*Pionus chalcopterus*).

**History:** Numerous birds of a flock with various parrots died spontaneously or after a short episode of unspecific symptoms.



Section of liver from a bronze-winged parrot. (HE, 4X)

**Gross Pathology:** Necropsy revealed hepatomegaly and multifocal randomly distributed white foci in the liver. Spleen and kidneys were swollen and moderate numbers of adult *Ascaridae* were present in the duodenum.

**Laboratory Results:** PCR tested negative for avian bornavirus. Ziehl-Nielsen staining of a liver imprint failed to identify acid-fast bacteria.

**Histopathologic Description:** Liver: The liver architecture is multifocally disrupted by randomly distributed areas up to 0.1 cm in diameter characterized by loss of cellular detail, karyorrhexis, karyolysis, and deposition of cellular debris (necrosis). Foci are surrounded by moderate numbers of degenerated and viable heterophils and macrophages. Occasionally hepatocytes and Kupffer cells contain intracytoplasmic grey indistinct granular structures. Increased numbers of aforementioned cells are also present within the sinusoids. Multifocally, most often surrounding bile ducts are moderate numbers of mainly lymphocytes, plasma cells, and fewer macrophages. Multifocally, bile ducts are minimal hyperplastic

and occasionally ectatic. Additionally, the liver is diffusely congested.

Giemsa staining revealed multifocal dark purple cytoplasmic inclusions in both hepatocytes and macrophages.

**Contributor's Morphologic Diagnosis:** Liver: Hepatitis, chronic-active, multifocal, random, necrotizing and granulomatous, moderate to severe, with intracellular bacteria consistent with *Chlamydophila psittaci*, bronze-winged parrot (*Pionus chalcopterus*), avian.

**Contributor's Comment:** The members of the order *Chlamydiales* are obligate intracellular, gram-negative bacteria depending on the host cells energy resources (ATP). They have a biphasic life cycle with the infectious form that enters the cell (elementary body, EB), the intracellular, metabolic active, replicating form (reticulate body, RB) and the intermediate body (IB) with morphologic characteristics between EB and RB.

Infection with *C. psittaci* occurs primarily via inhalation, ingestion or conjunctival exposure to contaminated feather dust or faeces. The bacteria spread to lung, air sacs and pericardial sac within few hours. EBs attach to microvilli of the host cell membrane and are internalized via invagination.<sup>4</sup> Bacteria-containing endocytic vesicles are called inclusions. They proceed to the nuclear area where EBs differentiate into RBs. A role of snapin (a part of the SNARE complex) connecting chlamydial inclusions with the microtubule network by interacting with both IncB and dynein has been proposed.<sup>1</sup> RBs replicate via binary fission and reorganize, through IBs, into new EBs. New EBs are released from host cell via cell lysis or exocytosis. In high pathogenic serotypes the inclusion membrane degrades mainly during the active multiplication, releasing the bacteria into the cytoplasm, leading to cell lysis.<sup>7</sup> The rapid multiplication leads to bacteraemia within 48 hours. Infectious EBs are spread via cloaca and/or nasal turbinates into the environment.

Typical clinical findings include diarrhoea and excretion of green to yellow-green urate. Severely affected birds may become anorectic and produce sparse, dark green droppings, followed by emaciation, dehydration and death.

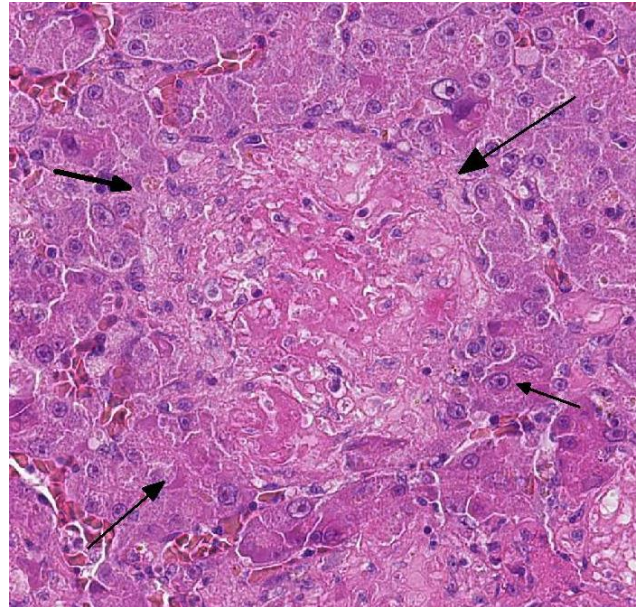
Typical gross findings are hepatomegaly and splenomegaly with or without necrotic foci, often accompanied by fibrinous airsacculitis, pericarditis and peritonitis and serous to purulent conjunctival exudate.

The genus *Chlamydophila* contains six species: *C. abortus* (ruminants), *C. caviae* (guinea pigs), *C. felis* (felidae), *C. pecorum* (ruminants, swine, koalas), *C. pneumonia* (humans, marsupialia and amphibia) and *C. psittaci* (birds). *Chlamydophila* spp. are known to infect at least 469 domestic, free-living or pet bird species in 30 orders.<sup>3</sup> Of the nine known “outer membrane protein A” (ompA) subtypes of *C. psittaci*, seven are known to naturally infect birds (A – F and E/B) and two to infect mammals (M56 – muskrat and snowshoe hare, WC – cattle). Each avian serotype seems to be associated with a different group or order of birds.<sup>2</sup> Furthermore, strains of *C. psittaci* fall into two general categories: highly or less virulent strains. Highly virulent strains or toxigenic strains (e.g. serotype D) are isolated most often from turkeys and occasionally from clinically inapparent wild birds. In natural and experimental hosts, they are responsible for a rapid and fatal disease progression characterized by extensive vascular congestion and inflammation of vital organs. Veterinarians and poultry workers are especially at risk of becoming infected with serovar D strains. Strains of low virulence (e.g. serotype B and E) cause slowly-progressing epidemics with a mortality rate of less than 5% and neither develop severe vascular damage nor severe clinical signs.<sup>6</sup> They are routinely isolated from pigeons and ducks. Chlamydiosis may be accompanied by concurrent infection with *Salmonella* spp, especially in pigeons, ducks and some psittacine birds. Avian strains can infect humans and may lead to severe pneumonia. However, cases of human-to-human transmission rarely occur. Immunity to chlamydia is generally poor and

short-lived. Older birds are often more susceptible to clinical signs than younger ones.

### JPC Diagnosis:

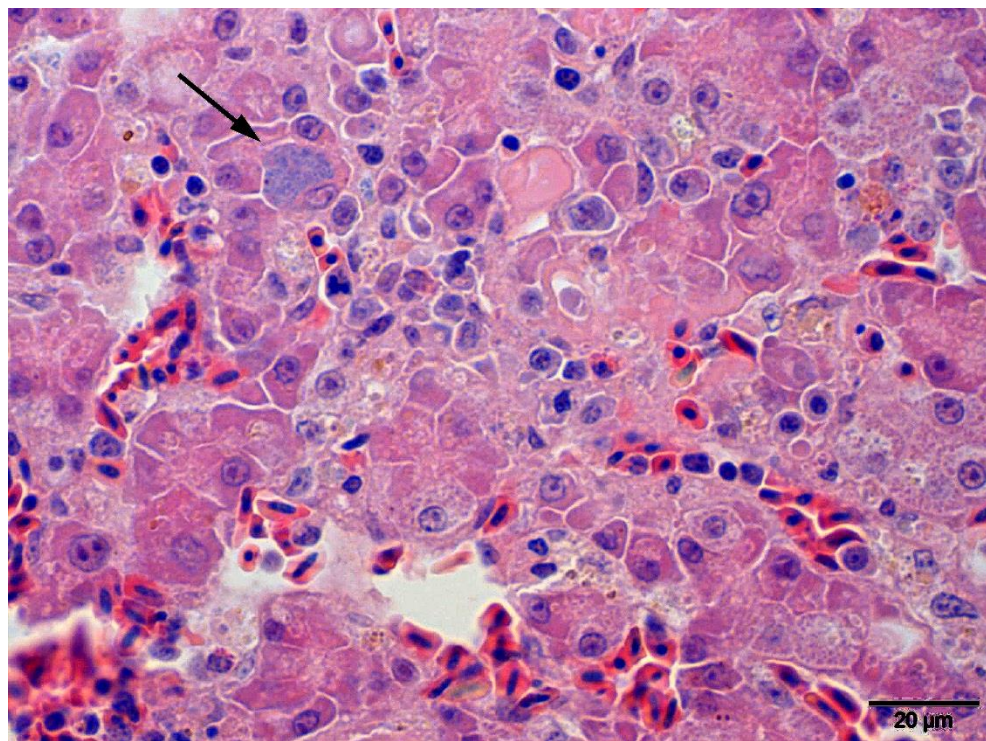
Liver: Hepatitis, necrotizing, random, multifocal, moderate with diffuse hepatocellular degeneration.



Liver, parrot. Multiple foci of coagulative necrosis are scattered throughout the section. Older foci are infiltrated by low numbers of heterophils and macrophages. (HE, 144X)

**Conference Comment:** Multifocal and random foci of coagulative and lytic necrosis effacing hepatic parenchyma were the predominant histologic features observed by conference participants. In less affected/non-necrotic parenchyma, changes indicative of hepatocellular degeneration including hepatocyte swelling, pallor, and centrilobular to midzonal vacuolar change were also described. Bile ducts containing blood and inflammatory cells were noted; however, this finding was deemed incidental and, in this case, unrelated to the pathogenesis of *C. psittaci*. Conference participants rarely identified 1-2µm, pale basophilic to amphophilic, intracellular organisms in macrophages and hepatocytes, most consistent with *C. psittaci* organisms. Slide variation regarding presence of organisms, was noted amongst conference participants.

Although not available for this conference, participants discussed various histochemical stains including Macchiovello and Gimenez stains, to identify and/or better highlight organisms in *C. psittaci*-suspect tissues. Common postmortem findings in *C. psittaci*-infected birds include hepatomegaly and splenomegaly, both which were observed grossly in this case. Histologically, the spleen is another primary organ to identify lesions and organisms. This infection is very common in Amazon parrots, which will demonstrate clinical illness. Pigeons and doves are common carriers, but do not become ill; however, they may facilitate zoonosis.



**Liver, parrot. Rare hepatocytes are expanded by aggregates of basophilic bacterial consistent with *Chlamydia*. Many of the hepatocytes show evidence of cellular degeneration characterized by the presence of large granules within their cytoplasm. (HE, 20X). (Photo courtesy of: Department of Veterinary Pathology, Freie Universitaet Berlin <http://www.vetmed.fu-berlin.de/en/einrichtungen/institute/we12/index.html>)**

All genotypes / subtypes of *C. psittaci* can be transmitted to people and cause psittacosis (i.e. parrot fever), a disease originally named when infection was thought to be only transmitted from psittacine birds. Most cases of human psittacosis are related to contact with psittaciformes; however, it is also an occupational hazard for

those working in the poultry industry and others in close contact with different avian species.<sup>8</sup> Infection in humans can result in a wide range of disease from asymptomatic infections to mild influenza-like symptoms, to severe disease with pulmonary and extrapulmonary involvement including gastrointestinal, hepatic, cardiac and neurologic signs.

*C. psittaci* infection in poultry production operations is most commonly associated with turkey or duck farms, but has also been documented in chickens, more commonly broilers. In chickens, both the *ompA* genotypes B and the more pathogenic D can cause clinical infection consisting of respiratory symptoms. However, as described above for other avian species, respiratory symptoms are generally more severe in genotype D infected chickens and it may also result in anorexia and mortality. Additionally, genotype D shows significantly higher bacterial replication in infected tissues and may demonstrate systemic dissemination. It is found to be excreted in the cloaca and from the pharynx in infected chickens as seen in other avian species.<sup>9</sup>

Atherosclerosis is frequently seen in captive psittacine birds and is considered the most common vascular disease of birds. Interestingly, it has been associated with the presence of *C. psittaci* antigen in blood vessels indicating infection may be a risk factor for development of atherosclerosis.<sup>5</sup> However, the exact role of *C. psittaci* infection in the pathogenesis of atherosclerosis, regarding severity and strain, is unclear.

**Contributing Institution:**

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http://www.vetmed.fuberlin.de/en/einrichtungen/institute/we12/index.html

**References:**

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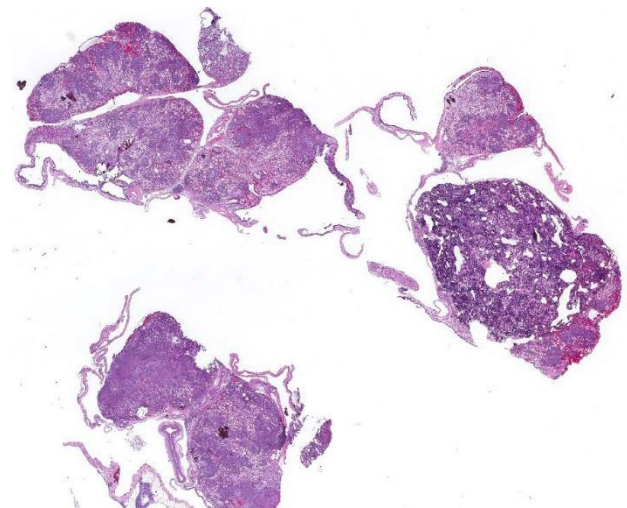
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**CASE III:** N2015-0172 (JPC 4066459).

**Signalment:** 14.5 year old, male, Colorado River toad (*Incilius alvarius*).

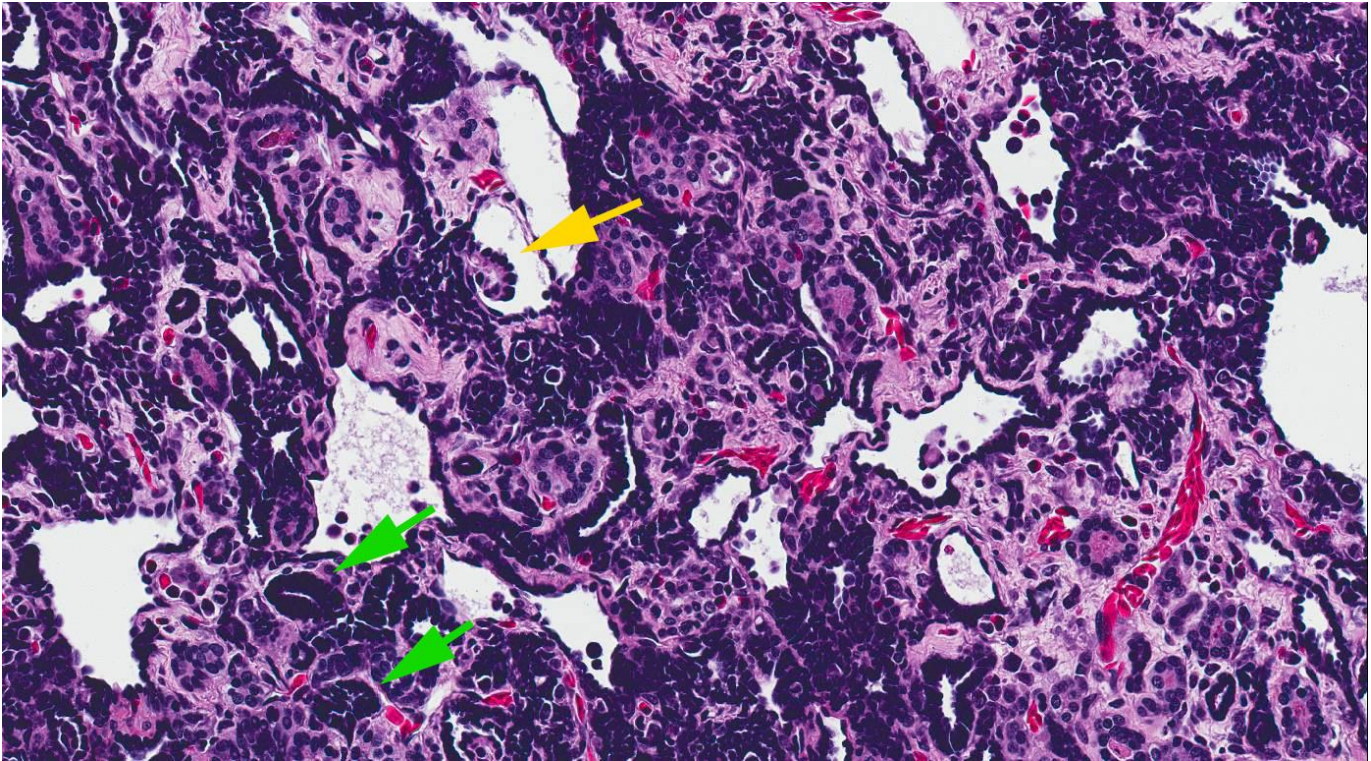
**History:** This individual was found dead with no premonitory signs. Post-traumatic necrotic hindlimb digits were removed approximately 7 months prior to death and the animal was reportedly normal in the interim.

**Gross Pathology:** Examined is a 243.6 g adult female Colorado River toad in fair body and good postmortem condition. There are numerous, discrete, round to oval wounds consisting of epidermal loss with red discoloration of the exposed tissue. Most are on the medial and ventral aspect of the left proximal hindlimb, left perianal area and caudal ventrum, but are also present on the right ventral brachium and antebrachium, near the elbow. Compression of the right forelimb results in expression of purulent material from one of these wounds. The distal aspect of all digits of the left hindlimb are absent, with only 1.0 mm to 2.0 mm remaining. The kidneys are diffusely mottled maroon and pink and have slightly nodular surface.



Multiple sections of kidney, adrenal, and interrenal glands are submitted. (HE, 6X)





*Kidney, toad. Renal tissue is multifocally effaced by ribbons of blastemal cells which often form tubule-like structures (green arrows), and often become cystic. In some cystic tubules a polypoid proliferation of blastema, resembling glomeruli, bulge into the tubular lumina (yellow arrow). (HE, 120X)*

There is a small amount of clear watery fluid in the ventral lymph sacs and a large amount of similar fluid in the coelomic cavity. Coelomic fat bodies are large and slightly pink to tan.

**Laboratory Results:** Aerobic culture (skin wounds): *Pseudomonas aeruginosa*, *Aeromonas* spp.

Anaerobic culture (skin wounds): *Fusobacterium* spp., *Bacteriodes* spp., unidentified Gram positive rods.

**Histopathologic Description:** Kidney and interrenal gland: Throughout the renal parenchyma and into the interrenal gland are variably sized, fairly discrete foci of inflammation composed of predominantly macrophages with fewer lymphocytes and granulocytes and admixed with abundant karyorrhectic debris. These granulomas often compress the surrounding parenchyma. Similar infiltrates multifocally expand Bowman's space and occasionally completely efface glomerular tufts. There are variably dense inflammatory infiltrates predominated by granulocytes

traversing the renal interstitium and are of highest densities surrounding granulomas. Tubules throughout the kidneys contain granular eosinophilic material admixed with karyorrhectic debris. Tubular epithelial cells frequently contain brown globular cytoplasmic pigment and there is rare intratubular crystalline material. This material is pale yellow and birefringent with polarized light (presumed oxalates). A focal ureteral branch contains abundant mineralization.

**Kidney:** There is a focal, unencapsulated, well-demarcated neoplasm composed of variably differentiated epithelial cells and blastemal cells set in a loose collagenous stroma. Blastemal cells have indistinct cell borders, scant to inapparent cytoplasm, and ovoid nuclei with hyperchromatic to finely stippled chromatin are present in dense clusters. Epithelial cells range from embryonal, with similar appearance to those above, forming ribbon-like strands and branching tubules, to more differentiated cuboidal to columnar cells forming mature, discrete tubules. These cells have distinct cell borders with a moderate

amount of eosinophilic cytoplasm that occasionally contains prominent granules. The nuclei are round to slightly ovoid, often basilar, with coarsely clumped chromatin. In some areas, tufts of cells protrude from primitive tubule walls into the lumen, resembling embryonic glomeruli. Mitotic figures are rare and only identified in blastemal populations. The stroma of this neoplasm is composed of loose aggregate of collagen interspersed with spindle to wavy cells. The interstitium contains few granulocytes, lymphocytes and macrophages.

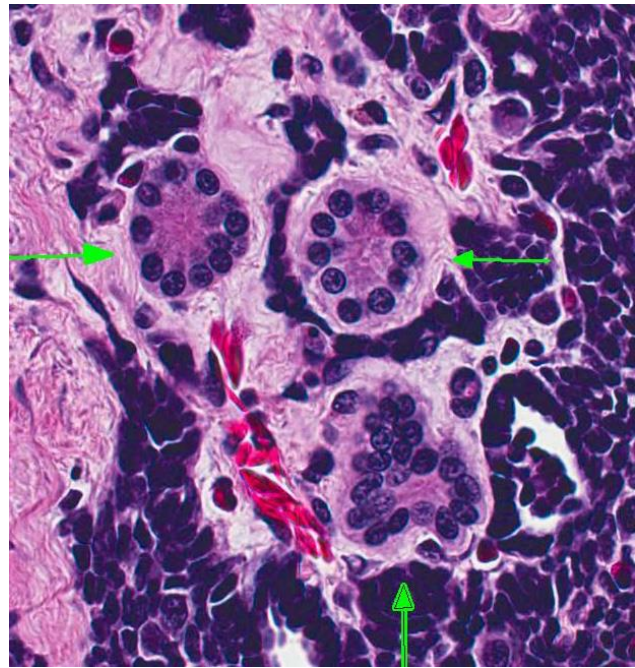
#### Contributor's Morphologic Diagnosis:

1. Kidneys and interrenal gland:  
Glomerulonephritis and adrenalitis, granulomatous, necrotizing, subacute, multifocal to coalescing, severe, with abundant intralesional acid-fast bacteria and renal architectural effacement
2. Kidney: Nephroblastoma

**Contributor's Comment:** Death of this Colorado River toad was the result of systemic mycobacteriosis. The most severe lesions were in the kidney, with granulomatous and necrotizing inflammation and containing abundant intralesional acid-fast and faintly Gram positive bacilli. There were numerous open skin wounds which, histologically, were chronic and also contained acid-fast bacteria. Given these findings, the wounds were considered to be the likely site of origin. Other tissues involved in the systemic infection included the interrenal gland, liver, heart and brain.

Mycobacteriosis in amphibians often presents as a disease of the integument, attributable to skin wounds and the organisms' common presence in water. Multiple non-tuberculous *Mycobacterium* species have been reported to cause disease in amphibians, including *M. marinum*, *M. chelonae*, *M. fortuitum*, *M. xenopi*, *M. abscessus*, *M. avium*, and *M. szulgai*; *M. marinum*, *M. xenopi* and *M. fortuitum* are reported as the most common isolates.<sup>6</sup> Dissemination to multiple organs is a common feature of amphibian mycobacteriosis, and is grossly identified as pale nodules within the parenchyma or grey patches on mesothelial surfaces. Associated inflammation is typically

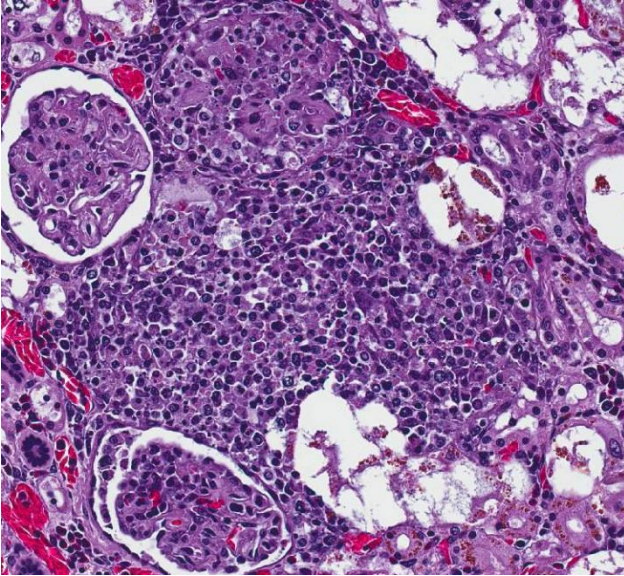
described as granulomatous, although the nature of the infiltrate depends on the stage of infection and can range from a mixture of granulocytes and macrophages in more acute lesions to more typical granulomas with macrophages centrally and lymphocytes peripherally in chronic lesions.<sup>1</sup> In this individual, the most severe lesions were in the kidney and consisted of discrete, but otherwise disorganized granulomas, suggestive of a subacute time course. Renal severity may be directly related to the location of the skin wounds, as hindlimb lesions in amphibians have been reported to spread directly to the kidneys.<sup>1</sup>



*Kidney, toad. Pre-existent chromaffin cells (green arrows) are scattered through the neoplasm. (HE, 200X)*

In addition to disseminated inflammation throughout the kidneys, there was a large, focal, unilateral renal neoplasm. The morphologic appearance of this tumor was consistent with a nephroblastoma (e.g. Wilms tumor, embryonal nephroma). Classically, nephroblastomas consist of three embryonal cell populations. These include an epithelial component which forms irregular tubules and immature glomerular tufts, a mesenchymal component forming a loose stroma, and an undifferentiated blastemal component dispersed throughout the tumor.<sup>4,5</sup> Differentiation of the mesenchymal component into muscle, bone and/or cartilage is common in

some species, but has not been reported in amphibians. In this case, there were few glomerular structures and the tumor was predominated by blastemal and epithelial cells, and no differentiation of the mesenchymal component. As in a previously described



*Kidney, toad. In other sections of the kidney, renal parenchyma is effaced by poorly-formed granulomas composed of large numbers of macrophages, admixed with fewer lymphocytes. (HE, 120X)*

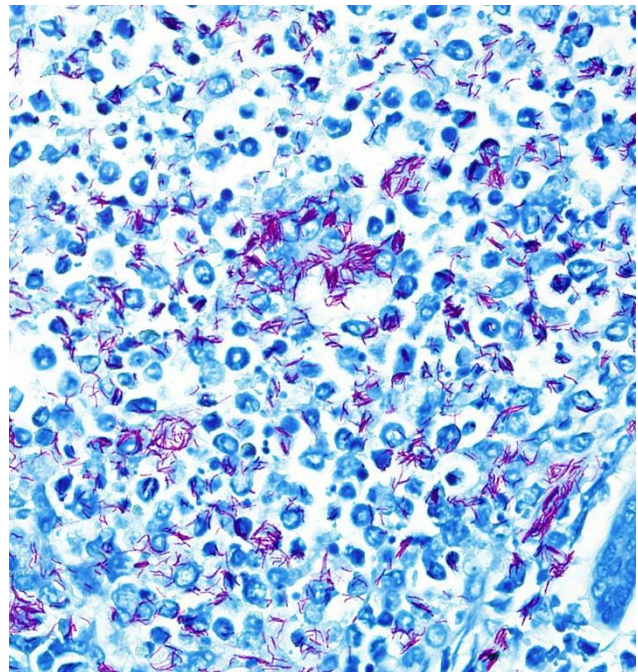
amphibian nephroblastoma,<sup>3</sup> tubules in this case were variably differentiated, with some neoplastic tubular cells containing eosinophilic granular cytoplasm. In mammals, birds and reptiles, nephroblastomas arise from the metanephric blastema, either from neoplastic transformation during nephrogenesis, or from persistent nephrogenic rests.<sup>5</sup> In amphibians and fish, which contain functional mesonephri throughout life, the tumor is thought to arise from mesonephric tissue. Rare in amphibians, nephroblastomas have been previously reported in an African clawed frog (*Xenopus laevis*), a fire-bellied newt (*Cynops pyrrhogaster*), a giant Japanese salamander (*Andrias japonicus*), and as an induced lesion in ribbed newts (*Pleurodeles waltl*).<sup>2,3</sup> Nephroblastoma can be confirmed in humans and other mammals by immunohistochemical identification of Wilms tumor protein 1 within neoplastic cells. This has been attempted in amphibians in only one of the previously reported cases, and neither tumor cells nor internal controls were positive.<sup>3</sup>

Immunohistochemistry was not pursued in this case.

**JPC Diagnosis:**

1. Kidney, testis, and interrenal gland: Nephroblastoma.
2. Kidney: Nephritis, granulomatous, multifocal to coalescing, moderate with intra- and extracellular acid fast bacilli.
3. Collecting duct: Nephrolith.

**Conference Comment:** In discussing the nephroblastoma, conference attendees noted the presence of well differentiated mesenchymal and epithelial elements, as well as embryonal elements. Islands of chromaffin cells were identified in one section of tissue, indicating the tumor had invaded and effaced the adrenal gland. Within the section of kidney affected by the mycobacterium infection, both degeneration and regeneration of renal tubule epithelial cells was

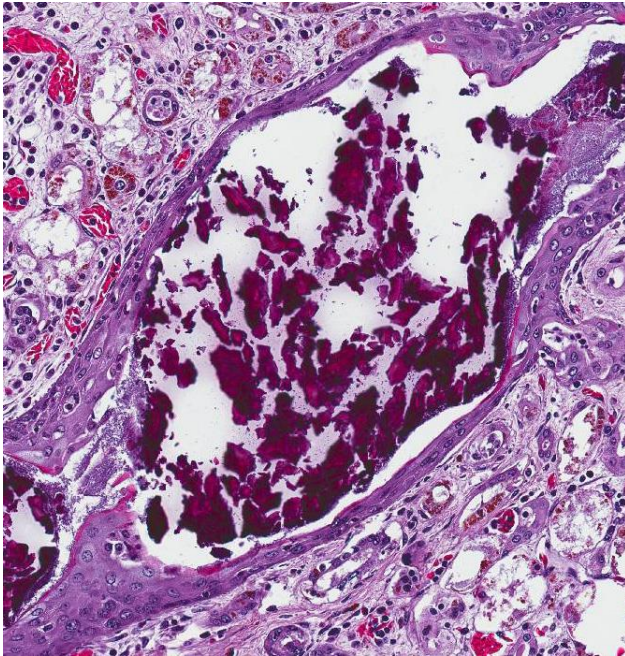


*Kidney, toad. Granulomas contain numerous 3-5um acid-fast bacilli (Ziehl-Nielsen, 100X) (Photo courtesy of: Wildlife Conservation Society, Wildlife Health Center, 2300 Southern Blvd., Bronx, NY 10460 <http://www.wcs.org>)*

noted, as well as the presence of cellular debris in ectatic tubule lumina. Overall, participants

agreed this was an excellent case and a challenging description.

In general mycobacteria can be classified into three groups: 1) Organisms that produce tubercles such as *M. tuberculosis* and *M. bovis*, 2) organisms that result in lepromatous inflammation such as *M. leprae* and 3) the atypical or non-tuberculous mycobacteria that may be opportunistic or primary pathogens; included in the third group are the mycobacterial organisms that infected the toad in this case. Many of the agents such as *M. marinum*, which is often the agent seen in amphibian infections, are zoonotic and can be important human pathogens in immunosuppressed and even non-immunosuppressed individuals. The organisms can be transmitted through direct contact with the animals or indirectly through the water.<sup>7</sup>



*Kidney, toad. Collecting ducts throughout the renal sections are filled with crystalline mineral (nephroliths). (HE, 110X)*

Internal organs commonly infected in amphibian mycobacteriosis include the liver, spleen, kidney as seen in this case, and intestines. Mycobacterial lesions may be misdiagnosed as lymphosarcoma in cases of amphibian mycobacteriosis but should be suspected in amphibians with granulomatous, lymphocytic or pyogranulomatous nodular inflammation in internal organs. Treatment is often ineffective and requires culling of affected individuals.

Mycobacterial infections are not uncommon in captive amphibians and can infect a number of species. Infections are less common in reptiles but have been reported in snakes, lizards, turtles and crocodiles. Lesion locations in reptiles include the liver, lung, spleen, kidney, oral cavity, joints and subcutis. In snakes, lesions may be seen in the oral cavity and lungs; in lizards disease is more commonly reported to be disseminated; and in chelonians mycobacteriosis may include pulmonary, hepatic, plastron and skin lesions.<sup>6</sup>

#### **Contributing Institution:**

Wildlife Conservation Society

[www.wcs.org](http://www.wcs.org)

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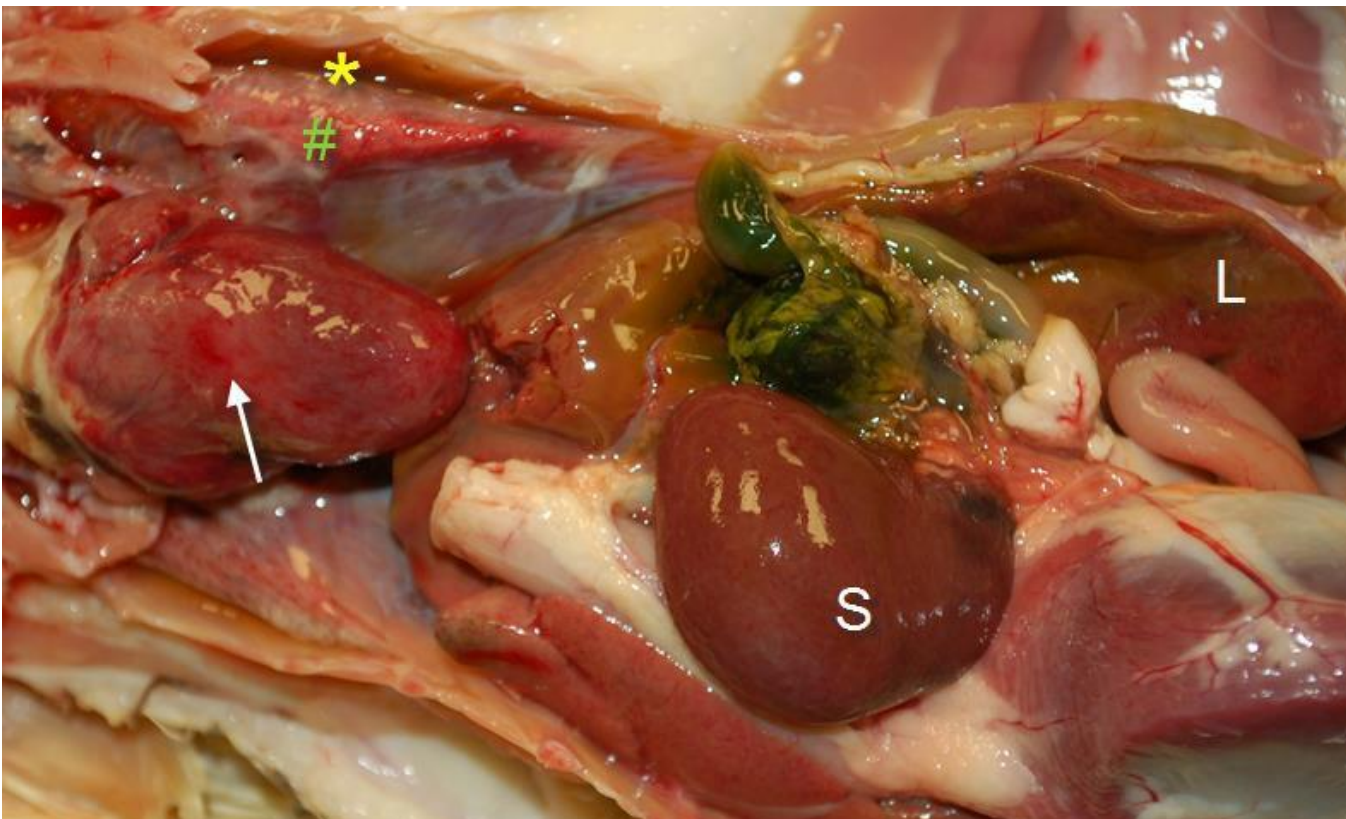
**CASE IV: A (JPC 4066233).**

**Signalment:** Six week-old, Welsh Harlequin Ducks (*Anas platyrhynchos domesticus*).

**History:** A group of eleven Welsh Harlequin ducklings hatched in Northern Ontario in late June were housed indoors until approximately 4-5 weeks of age, and then were allowed access to an outdoor enclosure during the day. Other than green watery diarrhea, the ducks were clinically normal. However in mid-August, at six weeks of age, one of the ducks became inappetent and

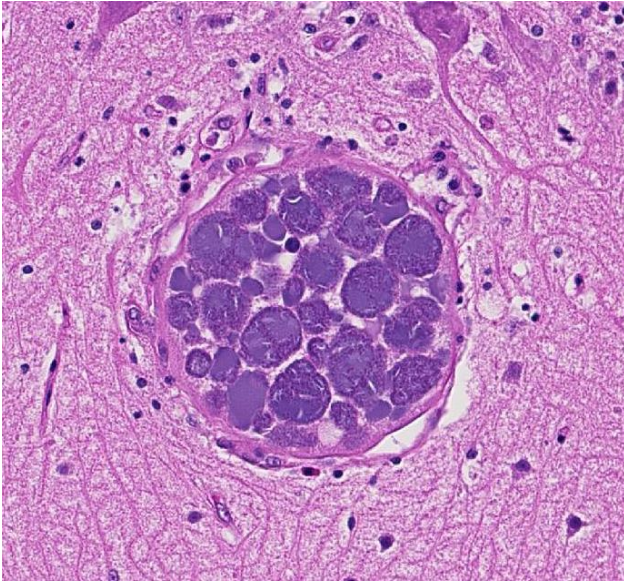
lethargic, exhibited labored breathing, and died overnight. In the following two days, three more ducks died, the last two exhibiting similar clinical signs as the first. In total, the owner lost 5 of the 11 ducks; two recovered following intensive supportive care. Chickens were also maintained on the same property but housed in a separate enclosure and suffered no morbidity or mortality. Pens were cleaned every 1-2 days; feed and water were always available and refreshed daily.

**Gross Pathology:** Two ducks were received for postmortem examination. Both ducks had similar findings and were in good body condition with good muscle mass, external and internal fat stores but the tissues were generally pale. There was mild generalized subcutaneous edema and focal hemorrhage in the caudal pectoral muscle. The lungs were very edematous, pink/purple, and pleural spaces of the lateral margins of the lungs contained small amounts of clear fluid. There was focal hemorrhage on the epicardium. The spleen was very enlarged and soft. The liver was



*There is focal hemorrhage on the epicardium (arrow). The spleen (S) was very enlarged and soft. The liver (L) was enlarged, tan/red and soft. There was mild generalized subcutaneous edema (\*) and focal hemorrhage in the caudal pectoral muscle (#). (Photo courtesy of: Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada <http://ahl.uoguelph.ca>)*

enlarged, tan/red and soft. The esophagus and proventriculus were empty; the gizzard contained grit and the intestine contained only a small amount of bile. Kidneys and bone marrow were pale.



*Cerebellum, duck: Scattered throughout all sections of the brain, endothelial cells are swollen up to 100um by megaloschizonts with numerous cytomeres. There is mild edema and gliosis of adjacent neuropil. (HE, 188X)*

**Laboratory Results:** No bacterial pathogens were isolated from Duck A spleen and Duck B heart blood. RT-PCR testing of lung/trachea and cecal tonsil pools for Avian influenza virus (AIV) and avian paramyxovirus-1 (APMV-1), and pooled brain/kidney for West Nile virus (WNV) and Eastern equine encephalitis virus (EEEV) were negative.

**Histopathologic Description:** BRAIN (Slide A): Variable numbers of large protozoal megaloschizonts containing enlarged host nuclei and numerous cytomeres and infrequently degenerate/ruptured megaloschizonts are present in the brain, occasionally within distended endothelial cells filling capillary lumens, infrequently rimmed by a small amount of hemorrhage with a few mononuclear cells and granulocytes in the surrounding parenchyma. The ruptured megaloschizonts are often surrounded by larger numbers of inflammatory cells, including multinucleated cells and released merozoites. Numerous small vessels are

infiltrated and surrounded by narrow cuffs of mononuclear cells and granulocytes. Hypertrophied astrocytes and reactive microglia are present within the parenchyma surrounding affected vessels, and also in close proximity to infrequent small foci of acute parenchymal necrosis.

**LIVER (Tissue not provided):** Within the liver, there is widespread multifocal to coalescent acute hepatic necrosis with mild to marked hemorrhage and hepatic dissociation. Sinusoids and vessels contain numerous red blood cells with eccentric compressed nuclei and round cytoplasmic structures morphologically compatible with protozoal gametocytes (Figure 2). Kupffer cells are enlarged and contain pale orange pigment, cellular debris and occasionally phagocytized erythrocytes.

#### **Contributor's Morphologic Diagnosis:**

**Brain:** Mild encephalitis with vasculitis and perivasculitis, mild multifocal necrosis with numerous intraparenchymal megaloschizonts

**Liver:** Marked acute multifocal to coalescent hepatic necrosis with numerous intraerythrocytic gametocytes and mild erythrophagocytosis

**Contributor's Comment:** The megaloschizonts with the enlarged host nuclei found only in the brain in these ducks are characteristic of *Leukocytozoon* spp.; *Leukocytozoon simondii* is the species that infects ducks and geese. The vector for *L. simondi* is the blackfly (*Simuliidae* spp.) and infective sporozoites carried in the salivary gland are introduced into the circulation of the bird at the time of the insect bite.<sup>1,3</sup> The sporozoites travel to the liver, enter hepatocytes and develop into first-generation meronts. These enlarge, go through multiple rounds of nuclear division, and form sections called cytomeres which are multinucleated. These further develop into uninucleate merozoites and multinucleate syncytia.<sup>1,3</sup> Some of the merozoites re-enter hepatocytes for a second round of merogony, while some merozoites and syncytia enter the circulation. The merozoites penetrate erythrocytes and develop into round gametocytes, and the intravascular syncytia

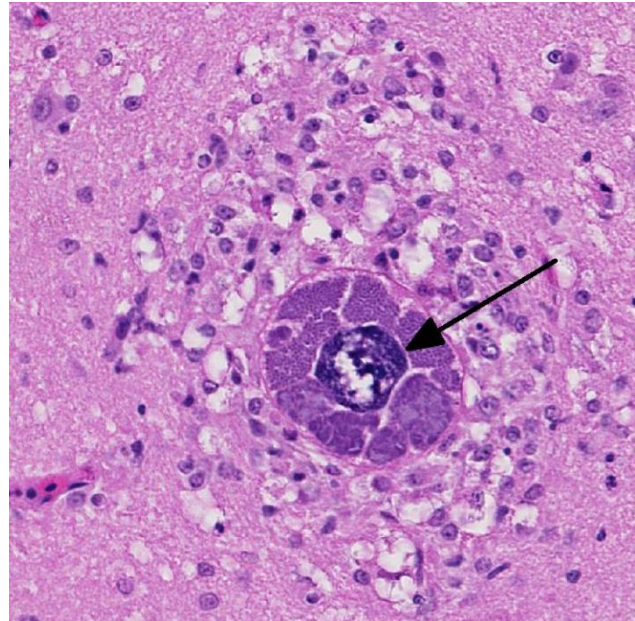
travel to various organs where they are phagocytized by reticuloendothelial cells, including macrophages, and develop into second generation meronts called megalomeronts or megaloschizonts because of their large size (100 to 200  $\mu\text{m}$ ).<sup>1,3</sup> A megaloschizont characteristically contains a markedly enlarged centrally located host nucleus and numerous cytomeres.<sup>1</sup> *L. simondi* megaloschizonts can be found in many tissues including brain, lung, spleen and heart.<sup>1</sup> When the megaloschizonts mature, they release large numbers of tiny merozoites that enter erythrocytes or leukocytes and develop into round or fusiform gametocytes.<sup>1,3</sup> The infective gametocytes are ingested by a biting fly, undergo sexual reproduction, produce a zygote that becomes a motile ookinete. Upon entering the small intestinal epithelium, the ookinete transforms into an oocyst, undergoes sporogony to produce sporozoites which then relocate to the salivary gland of the biting fly to complete the life cycle.<sup>1,3</sup>

The principal clinical effect of *Leucocytozoon* spp. infection is intravascular hemolytic anemia, thought to relate to the release of an antierythrocyte factor produced either by the meronts or their host cells rather than the primary mechanical destruction of the red blood cells by the protozoa, since the lowest hematocrit values occur before the parasitemic spike.<sup>4</sup>

In discussions with the referring veterinarian, the black fly population in the area appeared to be very low in early August, but in susceptible ducks, very limited exposure to infected blackflies is sufficient for the intravascular introduction of sufficient sporozoites to cause mortality. The prepatent period for *L. simondi* infection is approximately two weeks, so this fits well with the length of time from the initial release of the ducks into the outside enclosure and the onset of mortality. The producer has been advised that leukocytozoonosis is one disease that will have to be managed if they wish to continue to raise susceptible ducks in Northern Ontario.

**JPC Diagnosis:** Brain: Intraendothelial hemoprotezoal megaloschizonts, multifocal,

moderate, with intraerythrocytic gametocytes and minimal perivascular inflammation.

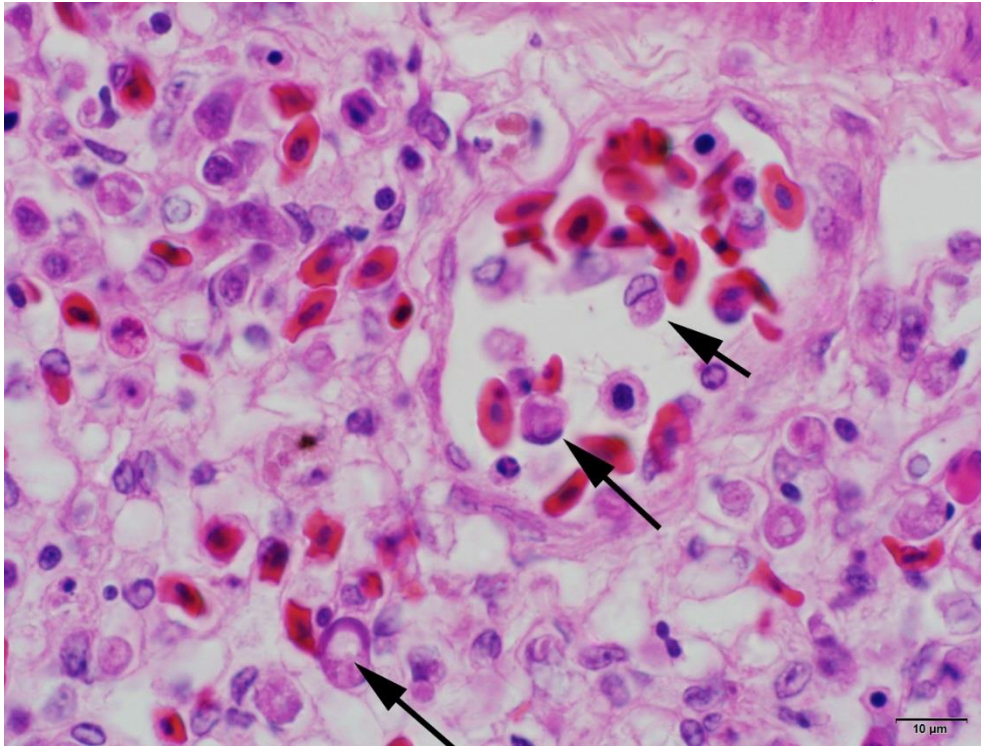


*Cerebellum, duck: Occasional parasitized endothelial cells exhibit hypertrophic nuclei surrounded by cytomeres. (HE, 188X)*

**Conference Comment:** The histologic features discussed during the conference were very similar to the contributor's histologic description. Slide variability was noted with some slides containing sections of pineal gland. Participants also discussed the ecology of avian hemoparasites and how it relates to host pathology. Infection with hemoparasites in the reservoir host is very common and often an incidental finding, resulting in minimal pathology. However, when an aberrant host is infected, the megaloschizonts can cause marked tissue damage, often in the liver and spleen, resulting in clinical signs and death in severe cases.

Avian blood parasites include three *Haemosporidia* genera which are closely related: *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. All are transmitted by a variety of biting insect vectors, which vary depending on the specific parasite, geographic location and distribution. The majority of bird species can become infected, but some species are more susceptible to infection than others (i.e. waterfowl, wild turkeys and penguins are commonly infected, but

infection is less common in migratory shorebirds).



**1**Cerebrum, duck: Erythrocytes contain clear vacuoles (gametocytes) which displace the nucleus to the periphery. (HE, 400X). (Photo courtesy of: Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada <http://ahl.uoguelph.ca>)

In general, *Plasmodium* and *Leucocytozoon* species are capable of causing more severe disease, and species of *Haemoproteus* are considered less pathogenic. Hepatomegaly and splenomegaly may be seen in infection with all three genera, but *Plasmodium* and *Haemoproteus* infection results in production of hemozoin pigment from digestion of hemoglobin, which can be seen in the liver, spleen and other organs, and may impart a black or brown appearance to the organs grossly. *Leucocytozoon* infection does not result in production of hemozoin pigment, so organs will not have the dark discoloration. A presumptive diagnosis can be made based on microscopic examination of a blood film, and the appearance of the parasite in red blood cells (RBC). *Leucocytozoon* results in the most dramatic change in RBC structure, with enlargement and elongation of RBCs and formation of hornlike extensions at each end of the cells; splitting of the nucleus may also be seen. With *Haemoproteus* and *Plasmodium*, less

dramatic changes are seen and include slight enlargement of the cells, lateral displacement of the nucleus, hemozoin pigment, and the presence of the small schizonts or gametocyte nuclei.<sup>2</sup> Because the different hemoprotozoa can cause similar lesions, both grossly and histologically, PCR is often necessary to reach a precise etiologic diagnosis.<sup>5</sup>

There are many species of *Leucocytozoon*, but not all are pathogenic. The species of *Leucocytozoon* which are pathogenic to wild birds include *L. simondi* (waterfowl), *L. marchouxi* (doves and pigeons) and *L. toddi* (raptors). The species which may cause disease in domestic birds include *L. simondi* (waterfowl; U.S., Canada, Europe), *L.*

*smithi* (turkeys; U.S., Canada), *L. macleani* (chickens; S.E. Asia), *L. struthionis* (ostriches; S. Africa), *L. schoutendeni* (chickens; sub-Saharan Africa, S.E. Asia) and *L. caulleryi* (chickens; south and S.E. Asia).<sup>1</sup>

In *Leucocytozoon* infection, gross lesions seen in infected birds include splenomegaly, hepatomegaly, tissue pallor and thinning of the blood. Histologically, capillaries may be distended by gametocytes with absence of host tissue reaction. The large megaloschizonts, which are often associated with small vessels, typically elicit a mononuclear inflammatory reaction but the meronts may or may not be associated with an inflammatory response. After the megaloschizonts rupture they may be filled with eosinophilic debris and mononuclear cells. Severe centrilobular hepatic necrosis may be seen along with periportal hepatitis and pigment laden macrophages / Kupffer cells. The enlarged spleen is congested, with loss of normal



architecture, and contains large macrophages distended with pigment and cellular debris. Lymphocytic and histiocytic infiltration may be seen in other organs such as the lung and myocardium.<sup>1</sup>

**Contributing Institution:**

Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada  
<http://ahl.uoguelph.ca>

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