CASE I: 2019A208 Case 2 (JPC 4130570).

Signalment: 4 months, female, Rhodesian Ridgeback, *Canis familiaris*, dog

History: The dog was presented on emergency for acute lethargy, anorexia and melena. Blood examination revealed increased CK and liver enzymes. On echography and clinical examination generalized lymphadenopathy was noticed. Soon the dog developed neurological signs with decreased consciousness, compulsive behavior and head pressing (forebrain disorder.) Also coagulopathy due to severe thrombocytopenia with increased coagulation times (PT and aPTT) was present. In 24 hours there was a progression of symptoms with neurological deterioration. Finally cardiac arrest developed with unsuccessful reanimation; the clinicians postulated hemorrhagic disorder, infectious or immune mediated encephalitis and metabolic encephalopathy as main differential diagnosis. Vaccination and deworming was up to date.

Gross Pathology: The liver was moderately pale, enlarged with a scant amount of fibrin strands on the capsule. There were several ruptures secondary to reanimation causing hemoabdomen. Spleen and lymph nodes were severely enlarged. The mesenteric lymph nodes were clearly edematous on cut surface. Many hemorrhages were seen at the serosal surfaces. Also severe edema of the gallbladder and melena was present. Dispersed small hemorrhages were present in the basal nuclei of the cerebrum and in the thalamus of the brain on cut surface.
Gallbladder, dog. There is marked edema of the gallbladder wall. (Photo courtesy of: The University of Ghent, https://www.ugent.be/di/di05/nl)

**Laboratory results:**

- Blood examination: increased CK 1199U/L (99-436), increased GOT/GPT and bile acids (no value available), thrombocytopenia 23 K/μL (148-484) and increased PT 18s (11-17) and aPTT 144s (72-102).

- Toxoplasmosis and neosporosis were excluded (IgG/M-determination.)

- Parvovirosis and angiostrongylosis were tested with a negative result.

**Microscopic Description:**

Multifocal in the midzonal to centrilobular region, areas of coagulation necrosis are present. This is characterized by swollen, eosinophilic, well circumscribed hepatocytes with a dark small, fragmented nucleus. In these areas loss of tissue architecture is present with pooling of red blood cells. Multifocal distributed, several hepatocytes and Kupffer’s cells contain large, intranuclear basophilic inclusions surrounded by a clear halo with margination of chromatin. In the sinusoidal spaces, there is a moderate increased amount of lymphocytes. There is a large subcapsular bleeding (secondary to liver rupture due to cardiac reanimation). On immunohistochemical staining for Canine adenovirus, there are numerous hepatocytes and Kupffer’s cells with positive staining nuclei.

**Contributor’s Morphologic Diagnosis:**

Severe multifocal midzonal to centrilobular hepatic necrosis with large basophilic intranuclear viral inclusion bodies, consistent with canine adenovirus type 1.

**Contributor’s Comment:** Non-enveloped dsDNA virus → family: Adenoviridae → genus: Mastadenovirus; there are 2 types.¹⁰

There is marked ecchymotic to suffusive hemorrhage of the mesentery and the mesenteric root. (Photo courtesy of: The University of Ghent, https://www.ugent.be/di/di05/nl)
Canine adenovirus - 1 (CAV-1) causes infection in numerous mammalian carnivores belonging to the Canidae, Mustelidae and Ursidae. In dogs, it causes infectious canine hepatitis (ICH) also called fox encephalitis or Rubarth’s disease. This virus causes a systemic infection mainly targeting the hepatocytes, endothelial and mesothelial cells. CAV-1 can cause a severe often fatal disease in juveniles. Death is very rare in animals older than 2 years of age. Clinical symptoms are variable, often vomiting, melena, fever and abdominal pain is present. In some cases nonspecific nervous signs occur. In a minority of cases there is acute death without preceding symptoms. During the recovery phase unil- or bilateral corneal edema can develop (“blue eye”). Years of widespread vaccination reduced the incidence of this disease in domestic animals in many countries to almost zero. In wildlife species CAV-1 is widespread, primarily as a subclinical infection but epizootic disease occurs. In foxes neurological symptoms are more frequently present than in dogs being the reason for its less popular name: fox encephalitis virus.

- CAV-2 causes a mild self-limiting infection of the upper respiratory tract and plays a role in the pathogenesis of infectious tracheobronchitis (ITB) known as kennel cough. Because of antibody cross-reaction it is used for CAV-1 vaccine production.

**Pathogenesis:**

Infection occurs through oronasal transmission. After reaching the oropharyngeal tonsils, tonsillitis develops accompanied by fever. The virus spreads to local lymph nodes and reaches the bloodstream through the lymph flow causing viremia which lasts 4-8 days. In a following phase the primary targets: hepatic parenchymal cells and endothelial cells (in all organs) get infected. Viral replication causes cell lysis and necrosis. Hepatic necrosis ensues at about day 7 (of experimental infection). Lesions in other organs are mainly secondary to vascular injury causing hemorrhage and edema. In a further stage, disseminated intravascular coagulation (DIC) can occur. This is caused by lysis of endothelial cells with activation of coagulation cascade and aggregation of thrombocytes. This causes increased consumption of clotting factors and thrombocytes resulting in consumptive coagulopathy. Several organ systems can be affected including the liver, kidney, lymph nodes, thymus, gastric serosa, pancreas and subcutaneous tissues. Edema of the gallbladder can be very prominent and is most likely due to increased vascular permeability secondary to vascular injury.

There are hemorrhages within both gray and white matter within the diencephalon. (Photo courtesy of: The University of Ghent, https://www.ugent.be/di/di05/ai)
Gallbladder edema is a pathognomonic sign at necropsy. The central nervous system can also be targeted causing an encephalitis with often inconspicuous lesions.

During the convalescent phase (day 7-21 days post infection) deposition of immune complexes (antigen-antibody) in the cornea can occur causing complement fixation and neutrophilic activation (type III hypersensitivity). The neutrophilic proteases cause diffuse hydropic degeneration of corneal endothelium and secondary stromal edema. The interstitial fluid accumulation causes deformation of corneal collagen fibers creating a blue-white aspect of the cornea (“blue eye”). The same mechanism happens in a small percentage of dogs 6-7 days after vaccination with modified live virus. In some cases inflammation proceeds and causes interstitial keratitis and permanent fibrosis. Alongside the corneal edema, anterior uveitis and glomerulonephritis can develop because of type III hypersensitivity.

**Gross findings:**
- Hepatomegaly; turgid and friable with sometimes a mottled yellowish aspect, fibrin strands at the capsular surface
- Edema and intramural bleeding of the gallbladder (pathognomonic)
- Enlarged, hemorrhagic, edematous lymph nodes
- Serosal bleeding (paintbrush lesions)

Gross lesions in other organs are inconstant:
- Hemorrhagic kidney infarctions (in young puppies)
- Enlarged, edematous tonsils (tonsillitis)

- Hemorrhages in the lungs, brain (midbrain and brainstem) and metaphyses of the long bones (ribs).
- Multifocal mucosal petechiae and subserosal hemorrhage
- Corneal edema (“blue eye”)

Liver, dog: A section of liver is submitted for examination. Upon close subgross inspection, there are areas of pallor and hemorrhage scattered throughout the section, as well as acute hemorrhage overlying the capsule. (HE, 7X)

**Histological findings:**

Liver:
- Centrilobular (periacinar) zonal necrosis (resembling acute hepatotoxicity.) In convalescence, hepatic regeneration occurs rapidly. After 2 weeks small foci of hepatocellular necrosis may be present. Foci of regenerating Kupffer’s cells may be present for another 2 weeks
- Intranuclear, large, basophilic inclusion bodies in endothelial cells, hepatocytes or Kupffer’s cells. Viral inclusions can be present after 4 days of experimental infection. At day 5-6-7 inclusions are most numerous, from day 8 numbers decline rapidly (in experimental infection)
- Fatty change
- Blood filled dilated sinusoids (because of loss of hepatocytes)
- Intact reticulin framework
- Mild leukocytic infiltration, mostly degenerating neutrophils

Other organs: Microscopic lesions in other organs are mainly secondary to endothelial damage.

- Brain: Hemorrhages of small vessels with rare intranuclear inclusions in endothelial cells which can be in relation with small foci of demyelination. Encephalitis can develop with very subtle lesions characterized by a mononuclear vasculitis with rarely more than a single perivascular monolayer of cells and hemorrhage.

- Lymphoreticular organs: Congestion, lymphocytolysis in lymphoid follicles and in the white pulp of the spleen, inclusions can be present in reticulum cells

- Kidney: Occasional intranuclear inclusion body in the glomerular mesangium cells or in the epithelial cells of the proximal convoluted tubule. Glomerulonephritis and secondary chronic interstitial nephritis can develop because of deposition of immune complexes in later stages.

- Lung: areas of hemorrhagic consolidation with hemorrhage, edema and fibrin formation in the alveoli with often inclusions in alveolar capillaries.

- Eye: corneal edema due to hydropic degeneration of corneal endothelium which can develop into interstitial keratitis and permanent fibrosis. Intranuclear inclusions can be present in a few degenerating endothelial
cells. There can be accompanying anterior uveitis characterized by accumulation of lymphocytes and plasma cells perivascular in the iris and ciliary body.\textsuperscript{8}

- In every organ macrophages with intranuclear inclusions can be present.\textsuperscript{5}

**Contributing Institution:**

University of Ghent
https://www.ugent.be/di/di05/nl

**JPC Diagnosis:** Liver: Hepatitis, necrotizing, multifocal, mild to moderate, with edema and numerous eosinophilic hepatocellular and endothelial intranuclear viral inclusion bodies.

**JPC Comment:** The contributor has given us an excellent and very comprehensive review of canine adenovirus-1 in the dog.

Liver infection with adenovirus have been a popular, although not especially diagnostically challenging, submission over the years in a number of species. This case marks the seventh time that CAV-1 has appeared in the WSC in the last forty years. Adenovirus in the liver of chickens (inclusion body hepatitis) has appeared a total of six times, with falcon adenovirus and bearded dragon (agamid) adenovirus appearing twice. (The complete list of adenoviral submissions over the years is well over a hundred, with pulmonary infections being the most common overall.)

In 1998, a case of CAV-1 in a skunk which manifested as fatal necrotizing hepatitis was reviewed in conference number 4, suggesting that this virus may cause disease in other wildlife species outside of canids.

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*Liver, dog: Within and at the periphery of foci of necrosis, hepatocellular and endothelial cell nuclei are expanded by an eosinophilic viral inclusion which is often surrounded by a clear halo. (HE, 400X)*
Although the conference results incorrectly identify skunks as canids (they are mustelids!), foxes, wolves and coyotes have certainly developed fatal disease associated with CAV-1. Skunks indeed have their own adenovirus (skunk adenovirus-1, first identified in 2015\(^5\)) which also causes fatal hepatitis (casting a bit of doubt over our 1998 case of CAV in a mustelid.) While adenoviruses are generally considered species-specific, skunk adenovirus-1 has been identified as causing pneumonia and trachieitis in pygmy hedehogs as well.\(^6\)

Several species of adenoviruses have also been identified by nested PCR in mustelids in the United Kingdom, but associated disease has not been ascribed to these viruses.\(^11\)

The most spirited discussion on this very classic case regarded the location of necrosis. While textbooks often discuss a characteristic pattern of centrilobular necrosis in affected animals, the typical pattern for viral hepatic infection would be expected to be random. Additionally, within many areas of necrosis, effete hepatocytes contain a basophilic stippling which some participants questioned might be an apicomplexan cyst. However, the material stained strongly positive with a Prussian Blue stain indicated that the material was iron-based. The cause of the ferrugination of occasional degenerate/necrotic cells was not clear.

References:


CASE II: HSRL-425 CVM-WU-2 (JPC 4038890).

Signalment: 3-year-old intact male feral domestic short hair cat (*Felis catus*)

History: An approximately 3-year-old 4.5 kg intact male feral, DSH (*Felis catus*) was presented to a veterinary hospital in Pomona, California. A good Samaritan used a live trap to capture the animal after she noticed excessive swelling in the nasal region. She was bitten during the capture process. The swelling was localized over the bridge of the nose and had a 3 cm ulcerated area that was bleeding excessively. Due to financial constraints and prognosis, the cat was euthanized and submitted for necropsy and rabies testing.

Upon initial examination, there was gross swelling at the bridge of the nose, and the eyes were nearly shut due to the severe conjunctivitis with abundant secretion. Dorsal to the nasal planum, there was an ulcerated area about ~3cm in diameter that had missing skin, covered by partially clotted blood mixed with necrotic debris.

Gross Pathology: The cat was in thin body condition with a large (3cm in diameter) ulcerated area on a severely swollen space-occupying lesion on the bridge of the nose. No gross abnormalities were found upon examination of internal organs. The cat was underweight and was ~10% dehydrated. A nasal swab was performed and stained with Diff Quik. Fungal organisms were visualized; however, narrow or broad based budding could not be identified. A tentative diagnosis of cryptococcosis was given.

Nasal planum has a large bulging mass (5cm in diameter) that expands and deforms the dorsal planum. The bulging mass has a superficial 3cm in diameter ulceration that connects with the nasal cavity. The ulcer is covered by partially clotted blood mixed with necrotic debris. The philtrum is intact. On cut section the mass is solid and occupies the nasal cavity replacing the turbinates and sinuses.

Laboratory results:
The brain was submitted *in toto* for rabies testing. Rabies testing was negative.

**Microscopic Description:**

Tissues submitted for histopathology included lung, liver, kidney, eye and portions of the nasal mass.

Skin from nasal area (from mucosa to haired skin): There is a locally extensive inflammatory area that extends to the deep limits of the examined sample. The submucosa is severely distorted and replaced by numerous round yeasts admixed with histiocytes, occasional multinucleated giant cells, and plasma cells. The yeasts vary from round with a thick wall to oval slightly pink transparent cells. These cells vary from 5-25 µm in diameter. The blastoconidia (asexual reproduction) is characterized by narrow based budding. The yeasts have a clear, thick capsule that gives the characteristic empty space around the yeast. The overlying epithelium is variably ulcerated with occasional acanthosis.

Nasal sections stained with PAS stain in addition to routine H&E staining show characteristic fungal organisms.

The eye was examined. There is severe suppurative inflammatory conjunctivitis with no fungal organism observed.

The other tissues show no significant changes.

**Contributor’s Morphologic Diagnosis:**

Morphologic Diagnosis: Nasal planum: Chronic, locally extensive, severe granulomatous rhinitis with superficial focal dermal ulceration and hemorrhage

**Contributor’s Comment:** Cryptococcus is an important dimorphic, basidiomycetous encapsulated fungal organism causing disease in humans and animals. Cats seem to be the most susceptible species.

It is an encapsulated fungus that replicates by narrow based budding (blastoconidia - asexual reproduction also called vegetative...
stage). The organism has also a sexual stage of reproduction called basidiospores rarely found in the natural cases. The infection occurs by environmental exposure and is not thought to be transmissible from one infected animal to another. The main environmental source is bird excreta, mainly pigeons.3,5,12

Cryptococcus are aerobic, non-fermentative organisms that form mucoid colonies on a variety of media. Virulence factors utilized by the yeast include its polysaccharide capsule, melanin, mannitol, and enzymes such as phospholipase, laccase and superoxide dismutase. Phopholipase and laccase are unique to C. neoformans and C. gatti and are thought to increase pathogenicity by interfering with the host immune response. The severity of the disease is the result of the combination of the virulence factors coupled with the immune status of the patient.1,3,12

The thick polysaccharide capsule interferes with the protective immune reaction against the yeast preventing successful phagocytic. There is some evidence to correlate the immune status of the patient to the inflammatory response against the organism. The most susceptible patients have T-cell deficiencies. In cats there is no breed or gender predisposition. Cats younger than 6 years appeared to be at higher risk. Retroviral infection in cats is not considered a risk factor. The most common presentations in cats include upper respiratory (like in this case), cutaneous, or central nervous system.1,3,5,10,11

Nose, cat. High magnification of one of the inflammatory nodules demonstrates numerous spherical to concave 6-15um yeasts with a 1-2um cell wall, amphophilic cytoplasm, and a 2-10 clear capsule, engulfed by numerous foamy macrophages. (HE, 400X)
There are about 40 different species within the genus of *Cryptococcus*. *C. neoformans* is the only pathogenic species. There are 4 species of *Cryptococcus neoformans* recognized which are separated in 4 different serotypes (A-D). The different types have different geographic distribution as well as virulence. *C. gatti* is considered being the most pathogenic.\(^5\)\(^9\) *C. gatti* (Cg) has been identified as an emerging infectious disease that not only affects immunosuppressed patients but also those with an intact immune response.\(^1\)\(^2\)\(^3\)\(^5\)\(^6\).

The lesions caused by *Cryptococcus spp* are composed of exclusively fungal organisms and minimal inflammatory response. The inflammatory reaction and fungal morphology is very characteristic however the differential diagnosis regarding the organisms includes coccidiodomycosis, *Candida*, and *Histoplasma*.\(^1\)\(^3\)\(^5\)\(^7\)

Histopathology, supported by silver stains (Gomori methenamine silver – GMS) or polysaccharide stains (periodic acid- Schiff - PAS) is the fastest and cost effective means of achieving a diagnosis when fungi are suspected. The diagnosis is made by the identification of the typical encapsulated yeast in cytology or histopathology. Its polysaccharide capsule positive to mucicarmine and Alcian blue stain is characteristic. The cerebrospinal fluid of affected patients can be stained with India ink to identify the organism. However alternative molecular diagnostic tests have been developed to achieve a definite diagnosis, including serum cryptococcal antigen that is thought to be the best way to reach a definitive diagnosis. Molecular...
diagnostic test are also available including PCR, amplified fragment length polymorphism (AFLP) analysis and Multilocus Sequence Typing (MLST).  

**Contributing Institution:**

College of Veterinary Medicine
Western University of Health Sciences
http://www.westernu.edu/xp/edu/veterinary/about.xml

**JPC Diagnosis:** Mucocutaneous junction, nose: Dermatitis, granulomatous, multifocal to coalescing, severe with numerous intra- and extracellular yeasts.

**JPC Comment:** In 1894, *Cryptococcus neoformans* was simultaneously identified as a human and juice pathogen by pathologist Otto Busse (from a granulomatous lesion in the tibia of a 31-year-old woman), and Italian scientist Francesco Sanfelipe (from peach juice). The true pathogenic nature of *C. neoformans*, however, was not realized until the 1980’s when immunosuppressed AIDS patients provided appropriate venue for its pathogenic abilities and a tragic opportunity for widespread research.

The basics of *Cryptococcus* infection are well known, and reviewed by the contributor above. In the last decade, a number of new insights have been gained into the pathogenic mechanisms of cryptococcal infection.

Size in important in many phases of cryptococcal infection. Infection is most likely the result of inhalation, but not of the yeasts of the size seen in this case (6-15um) which are too large for inhalation into the deeper airways of the lung (usually 5um or less). Infection more likely occurs from desiccated cells or spores, which set the stage for latent infection which is stimulated to growth by immunosuppression later in life.

Another instance in which yeast size impacts pathogenesis is the formation of atypical yeast forms which helps cryptococcus in establishing latent infection or perpetuating its disease-causing growth phase. Unusually small forms, known as metabolically inactive “drop” or “micro” cells measure 2-4um and possess a thickened cell wall, making them attractive for phagocytosis by macrophages and likely important in latent infection. Another atypical form resides at the opposite pole of the size spectrum – “titan” cells, polyploid cells which exceed 12um in diameter. These cells have a highly crosslinked resistant capsule and also a thickened cell wall with chitin to assist in phagocytosis evasion. The production of host chitinases need to break down these forms appears to be detrimental to the overall inflammatory response.

Two distinct pathogenic species, *Two distinct species, Cryptococcus neoformans* and *Cryptococcus gatti* exist within the genus since 2000. They differ in host requirements, with *C. neoformans* (and its subspecies *C. neoformans* var. *grubii*) causing disease in immunosuppressed hosts, and *C. gatti* infecting immunocompetent animals. One of the commonly infected animals in Australia, unfortunately, is the koala, which lives among species of eucalyptus trees which naturally host the sexual phase of the fungus. In some parts of Australia, especially on the eastern coast, colonization rates of of 94-100% occur in koalas, and may act as a reservoir and amplifying host. Affected koalas present with pneumonia and minoencephalitis, but many koalas possess subclinical infections which provide a resource for studying latent infection.
One of the attendees from the National Zoo mentioned that animals that had been treated long term with antifungal agents may have greatly reduced to absent capsules, and that additionally, this fungus may be pigmented. *Cryptococcus* also has an organelle known as a microsome, which allows it to metabolize xanthine and urates, which facilitates its survival in bird feces.

**References:**

CASE III: 18-2108 WSC #3 HE (JPC 4117383).

Signalment: 3-1yr female red golden pheasant (Chrysopholus pictus).

History: A pheasant flock of 50 birds was experiencing respiratory signs, including nasal congestion, puffy eyes, and ocular discharge. Twelve pheasants died over approximately one month. The owner purchased an additional 12-16 new pheasants from Michigan and Ohio. These new pheasants seemed thin, weak, and lethargic. They were introduced directly into the original flock without a quarantine period. There were no further deaths in the original flock, but eight of the new pheasants died within a three-week period. This golden pheasant was submitted for necropsy together with two Impeyan pheasants for necropsy, both of which had a severe mucopurulent infraorbital sinusitis and rhinitis.

Gross Pathology: The pheasant weighed 470g and was in good body condition. There were innumerable firm yellow to reddened nodules each up to 3mm in diameter covering the serosal surfaces of the distal segment of the gastrointestinal tract, affecting the ceca most severely but with additional nodules over the colorectum, ileum, and distal jejunum. These nodules were also present in the mucosa of the ceca. No gross lesions were identified in the nasal cavities, trachea, or lungs of this pheasant.

Laboratory results:

Aerobic and Mycoplasma cultures from the affected sinuses of the two Impeyan pheasants yielded no growth.

Microscopic Description:

Cecum, ileum, and colorectum: Many well-circumscribed nodules, each up to 3mm in diameter and becoming confluent, expand the submucosa, muscularis externa and

Trachea, ceca, colon, golden pheasant. There are numerous <3mm nodules within the muscularis and serosa of the cecum and colon of this bird. (Photo courtesy of: Connecticut Veterinary Medical Diagnostic Laboratory at the University of Connecticut, http://cvmdl.uconn.edu/)

Trachea, ceca, colon, golden pheasant. Multiple sections of trachea, cecum, and colon are submitted for examination. At subgross magnification, numerous nodules may be seen within the sections of gut, and larval and adult ascarids may be viewed within them. (HE, 7X)
serosa, and are sometimes pedunculated from the serosal tissue. The cecum is most severely affected. Nodules consist primarily of spindle cells with a large volume of pale eosinophilic cytoplasm and indistinct cell borders, having large round to oval nuclei with vesicular chromatin and a prominent nucleolus. Cells are arranged in whorls, and sometimes interlacing streams and bundles. Nodules are infiltrated by mild to moderate numbers of macrophages and small lymphocytes with fewer plasma cells, and are surrounded by a thin collagenous capsule. Many of these nodules contain nematode parasites of 0.3mm in diameter in cross section or tangential section. Nematodes are characterized by a thin smooth cuticle with lateral alae and a thin hypodermis, large lateral chords, and thick polymyarian, coelomyarian musculature. A pseudocoelom contains a distinct digestive tract with columnar enterocytes, and non-embryonated eggs are present in a uterus in some sections. Some of these nematodes are degenerate and fragmented and are surrounded by increased numbers of leukocytes, including multinucleated giant cells and heterophils. There is moderate congestion of vessels within some nodules with occasional extravasation of erythrocytes, and there are occasional aggregates of hemosiderin-laden macrophages. Additional nematodes are rarely present in the cecal lumen.

There is some variation among slides and sections.

TRACHEA: There is circumferential erosion of the tracheal epithelium, and the tracheal mucosa is covered by a thin pseudomembrane composed of fibrillar eosinophilic material (fibrin) admixed with heterophils, sloughed degenerate cells, and small numbers of erythrocytes. The epithelium contains large syncytial cells with up to 25 nuclei, which slough into the tracheal lumen. Nuclei in syncytial cells often contain large hyaline eosinophilic to basophilic viral intranuclear inclusions that peripheralize the chromatin. Individual epithelial cells sometimes contain amphophilic to basophilic inclusions. Cilia are absent from most of the remaining epithelium which is attenuated to a low cuboidal level in certain areas, while in other areas there is a reparative process where epithelium is 4-5 cells thick and disorganized, and nuclei are large with prominent nucleoli. The subjacent
submucosa is expanded by edema and low to medium numbers of heterophils, macrophages, and lymphocytes; mucus glands are not seen.

**Contributor’s Morphologic Diagnosis:**

Cecum, ileum, and colorectum: Atypical nodular mesenchymal proliferation, submucosal, mural and serosal, severe, with granulomatous inflammation and intralesion adult nematodes, morphology consistent with *Heterakis* spp.

Trachea: Tracheitis, fibrinonecrotizing, diffuse, severe, with epithelial syncytia formation and intranuclear viral inclusion bodies, consistent with gallid herpesvirus-1.

**Contributor’s Comment:** The microscopic findings in this pheasant are characteristic for gallid herpesvirus type 1 (GaHV-1), the cause of infectious laryngotracheitis, and for *Heterakis* infection.

GaHV-1 is a member of the genus *Iltovirus*, subfamily *alphaherpesviridae* of the *herpesviridae* family. The disease was first described in poultry in the United States in 1925, and in pheasants in 1931. Pheasants, pea fowl, and very young turkeys are also susceptible, while quail, guinea fowl, and non-galliform birds are resistant. Birds that survive become lifelong carriers that may continue to shed, and wind can carry the virus between backyard flocks and larger production facilities. The virus is susceptible to disinfectants and to light exposure, but can survive in moist litter for 4 days or in dry litter for 20 days. Naturally infected birds develop clinical illness 6-14 days post-exposure. Viral replication is limited to the respiratory epithelium; latency is achieved by infection of the trigeminal ganglion. Birds affected in an epizootic can develop bloody mucus throughout the upper respiratory tract and experience severe respiratory distress and high mortality, while enzootic forms can cause varying degrees of mucoid tracheitis, conjunctivitis, and sinusitis, with lower mortality.

Though this bird did not have significant rhinitis or sinusitis on gross anatomic examination, the two Impeyan pheasants submitted concurrently did have marked exudates in their upper respiratory tracts.
and characteristic intranuclear inclusions in sloughed syncytial cells were identified on histopathology on all three birds. These inclusions are pathognomonic and can be identified with hematoxylin and eosin stain or with Giemsa.3 Where GaHV-1 is suspected but inclusions are absent, viral isolation from chicken embryo liver cells, detection of GaHV-1 antigen by direct fluorescent antibody or immunohistochemical staining, or GaHV-1 DNA detection by PCR are appropriate.3 Serology is not generally advised due to cross-reactivity with vaccine-induced antibodies.3 Differential diagnoses for affected birds include infection by avian paramyxovirus (Newcastle disease), avian influenza, adenovirus infection, and avian coronaviruses.3

*Heterakis* spp infection is common in many galliform species. The life cycle is direct but earthworms may serve as paratenic hosts, making eradication of infection from birds on pasture or earthen lots exceedingly difficult.1,8 *Heterakis dispar* has been reported in pheasants and is relatively non-pathogenic, while *H. gallinarum* and *H. isolonche* have both been reported to cause typhlitis in pheasants. *H. gallinarum* is infrequently a major primary pathogen in poultry, however it serves as a transport host for the protozoal parasite *Histomonas meleagrisidis*, which causes blackhead disease in turkeys and sometimes in chickens. However, in pheasants, there are no reports of histomoniasis, and disease is due to the nematode itself.

*Heterakis isolonche* is usually more pathogenic and has been reported to cause...
high mortality in pen-reared pheasants.\textsuperscript{8} It causes extensive nodule formation in the distal digestive tract due both to granuloma formation and marked proliferative host response, with the ceca most severely affected. These nodules sometimes rupture, causing peritonitis/coelomitis.\textsuperscript{4} Second-stage larvae of \textit{H. isolonche} are released from eggs in the gizzard, migrate to the ceca, then penetrate the mucosa\textsuperscript{1}. In the submucosa, the larvae spark a proliferative response resulting in the yellow to pink to dark brown nodules seen grossly.\textsuperscript{1} The worms frequently reside in the nodules.\textsuperscript{1} Previous studies have variably characterized the nodules associated with these worms as granuloma, fibroma, fibrohistiocytic neoplasia, or leiomyoma, indicating the variability in response and limited studies attempting to characterize the proliferating cells.\textsuperscript{4,5,6,9}

While lesions are sometimes reported as benign neoplasia, there is a case report of metastatic lesions of neurofibroblastic origin developing in the lung and liver of a 9-year-old ring-necked pheasant (\textit{Phasianus colchicus}).\textsuperscript{7}

Some consider \textit{H. isolonche} to be an example of a nematode that induces neoplasia.\textsuperscript{1} \textit{Spirocerca lupi} is known to induce fibrosarcoma and osteosarcoma in the esophagus of dogs.\textsuperscript{12} In our case, trichrome stain revealed no collagen among the proliferating fusiform cells, and immunohistochemical staining for smooth muscle actin and CD68 for cells of macrophage lineage were both negative, with appropriate staining of muscularis externa and individual inflammatory cells serving as appropriate internal positive controls, respectively. \textit{H. gallinarum} and \textit{H. isolonche} are very similar worms, and are

\textbf{Nodules are composed of tight interlacing bundles of spindle cells with widely scattered lymphocytes and macrophages. (HE, 131X) (HE, 22X)}
usually differentiated at the gross level. *H. isolonche* is slightly longer than *H. gallinarum*, and its spicules are more symmetrical than those of *H. gallinarum*. In this case, intraluminal worms were not recognized grossly and are rarely seen on histologic section, features consistent with the life cycle of *H. isolonche* and not of *H. gallinarum*.

Of relevance to this case, there are multiple case reports describing increased pathogenicity in certain pheasant species including golden pheasants, though these cases do not describe the extensive involvement of the tunica muscularis externa or serosa of the ceca and surrounding digestive tract as seen in this bird. A cause for the increased disease severity in golden pheasants has not been elucidated, however parallels between the migrations of strongyles in equids, of hookworms in Southern fur seals, and of *H. gallinarum* in other galliform species may provide further insight.

**Contributing Institution:**
Connecticut Veterinary Medical Diagnostic Laboratory at the University of Connecticut,
http://cvmdl.uconn.edu/

**JPC Diagnosis:** 1. Trachea: Tracheitis, necrotizing and heterophilic, moderate with multifocal ulceration, epithelial intranuclear viral inclusion bodies and viral syncytia.

2. Cecum, large intestine, coelom: Typhlitis, colitis, and coelomitis, granulomatous, multifocal, moderate, with nodular spindle cell proliferations, and adult and larval ascarids.

**JPC Comment:** The participants agreed that this was a challenging descriptive exercise, with two distinct entities, as well as an adult nematode to describe. While most WSC submissions allow participants to focus on a single pathologic agent and process, there are two excellent unrelated conditions ongoing in this submission.

One of the characteristics of infectious laryngotracheitis and a number of other

*Cecum, golden pheasant. A tangential section of an intranodular ascarids exhibits lateral alae, a thin cuticle, lateral chords, polymyarian coelomyarian musculature, an intestine with numerous columnar uninucleate epithelium and multiple cross-sections of gonad. (HE, 219X)*
alphaherpesviruses, (to include herpes simplex virus 1 and 2, anatid herpesvirus-1, and a number of others - but certainly not all) is the formation of characteristic syncytial cells, (prominent in this particular specimen.) In ILT, syncytial cells are commonly seen in the exudate overlying the remnants of the intact mucosa; these are virally infected, mutated, and degenerating cells which have lost contact with adjacent epithelium, a process that usually results in epithelial death. In alphaherpesvirus infection, membrane fusion is a key element of initial cell entry and lateral spread of virus in tissues. It requires four envelope proteins, gB, gD, gH, and gL. When mutated, the gB and gK genes confer hyperfusogenicity on HSV and an accelerated and exaggerated process result in the formation of syncytial cells. Essentially, the formation of syncytia is a normal process associated with viral transfection gone haywire as a result of mutation.

The contributor has also done an excellent job in discussing the unique proliferative change resulting from infection with *H. isolonche* in pheasants. This precise histogenesis of these nodules have not to date been elucidated. Immunostaining for smooth muscle, desmin, IBA-1, and histochemical staining for collagen failed to identify a smooth muscle or histiocytic origin for these nodules, and a literature review fails to elucidate their origin as well. It is good to have some things that modern science cannot explain.

There are a few other species of *Heterakis* species of note. *Heterakis gallinarum* in itself does not cause any significant disease, but is a host for *Histomonas melagridis*, a protozoan parasite which causes necrotizing typhlitis and hepatitis in turkeys and other poultry species. *Heterakis bonasae* infects the ceca of ruffed grouse and bobwhite quail and heavy infections may result in ill thrift and death. *Heterakis spumosa* is largely a historical pathogen in laboratory rodents which infects the cecum or colon and is not considered pathogenic.

The contributor mentioned parasites which may result in neoplasia in their host. Other examples include *Opisthorchis felineus* and *Clonorchis sinensis* which cause cholangiocarcinoma in cats and humans; *Cysticercus fasciolaris* which was documented to cause hepatic sarcoma in rats; *Trichosomoides crassicauda* which causes of the urothelium in rats; and *Schistosoma haematobium* a well-known cause of transitional cell carcinoma of urinary bladder in humans.

**References:**


**CASE IV: Rab 51 (JPC 4118093).**

**Signalment:** Adult (age unknown), female entire, wild European rabbit, *Oryctolagus cuniculus*.

**History:** This rabbit is from a group of wild rabbits shot for population control and subsequently submitted for post mortem examination for research and teaching purposes (institutional Ethics and Welfare Committee reference: CR240).

**Gross Pathology:** The lungs have a light brown to pink surface and a firm, gritty texture. A large number of coalescing granulomas that measure up to 1 mm in diameter expand the pulmonary parenchyma on cut surface (Fig. 1). Tracheobronchial lymph nodes have smooth, homogenous and cream capsular and cut surfaces and measure approximately 8x5x5 mm.

There are multifocal, randomly distributed, well demarcated, cream foci within the liver
parenchyma and level with or slightly raised from the capsular surface. These foci measure up to 2 mm in diameter (gross and microscopic findings confirmed concurrent *Eimeria stiedae* infection).

**Laboratory results:**

None available.

**Microscopic Description:**

Greater than 90% of the pulmonary parenchyma is expanded by a large number of multifocal to coalescing granulomas surrounding thick walled adiaspores. Adiaspores measure 200-300µm in diameter and have a wall which consists of a bright eosinophilic, approximately 3µm thick outer layer, a 30-40µm thick pale eosinophilic middle layer and a variably prominent, 2-3µm thick, basophilic inner layer. The adiaspore wall surrounds a foamy, variably pale basophilic core. Large numbers of concentrically arranged heterophils, macrophages, and epithelioid macrophages, small to moderate numbers of lymphocytes and plasma cells, moderate numbers of multinucleated giant cells (foreign body type, 10-50 nuclei) and variable numbers of fibroblasts surround the adiaspores. At the center of some granulomas are degenerate adiaspore remnants admixed with heterophils or a core of necrotic debris. Granulomas multifocally extend up to or raise the visceral pleural surface. Adiaspore walls are intensely and stain intensely with periodic acid-Schiff stain.

*Lung, rabbit. Numerous 1mm granulomas expand the pulmonary parenchyma. (Photo courtesy of: Department of Veterinary Medicine, The Queen's Veterinary School Hospital, University of Cambridge. Cambridge CB3 0ES, UK. [https://www.vet.cam.ac.uk]*)
Contributor’s Morphologic Diagnosis:

Pneumonia, granulomatous and heterophilic, chronic, multifocal to coalescing, severe; lungs, with adiaspores consistent with *Emmonsia crescens*.

**Contributor’s Comment** Adiaspiromycosis is associated with two causative agents: *Emmonsia crescens* and *Emmonsia parva*, which are saprophytic dimorphic fungi of the *Ajellomycetaceae* family. *E. parva* is distributed in small geographical areas in North America, Asia, Australia, and eastern Europe, has a small adiaspore diameter (10-20µm) and grows up to 40°C. *E. crescens* has a worldwide distribution, large adiaspores (up to 300µm), and grows up to 37°C.14

Following inhalation of conidia from soil, adiaspores develop and increase in size in host tissue which leads to granulomatous pneumonia in the host³. Small mammals such as rodents⁵,⁷ and mustelids⁹ are most commonly affected, but adiaspiromycosis has also been sporadically reported in larger mammals such as horses¹², deer¹¹, badgers¹⁰, foxes¹⁰ and dogs⁸. Adiaspiromycosis occurs rarely in humans and has a range of presentations from solitary pulmonary nodules to disseminated pulmonary disease and is occasionally fatal. A disseminated, extrapulmonary form has been described in immunosuppressed patients with acquired immunodeficiency syndrome and in one HIV-positive patient on immunosuppressive therapy following liver transplant.¹.

Adiaspiromycosis has been previously reported in cottontail rabbits (*Sylvilagus audubonii*) in New Mexico¹⁵ and is recognized in *Oryctolagus cuniculus* as a historic, occasional, pathogen of laboratory rabbits.

In this case, a wild European rabbit (*Oryctolagus cuniculus*) exhibited granulomatous pneumonia which was particularly marked both grossly and microscopically.⁶ All lung lobes were affected. Additionally, the cortex and medulla of the tracheobronchial lymph node were multifocally expanded by moderate numbers of adiaspores, with a similar histological appearance to those in the lungs,

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In this case, a wild European rabbit (*Oryctolagus cuniculus*) exhibited granulomatous pneumonia which was particularly marked both grossly and microscopically.⁶ All lung lobes were affected. Additionally, the cortex and medulla of the tracheobronchial lymph node were multifocally expanded by moderate numbers of adiaspores, with a similar histological appearance to those in the lungs,
and surrounded by large numbers of epithelioid macrophages, moderate numbers of multinucleated giant cells (foreign body type, 10-50 nuclei) and fewer heterophils. Adiaspores do not replicate within host tissue and so the extent and severity of this case was most likely related to inhalation of large numbers of conidia. Adiaspore morphology was considered most consistent with *E. crescens* due to the diameter.

*E. crescens* infection can be confirmed by microdissection of adiaspores and PCR amplification using *Emmonsia* specific primers followed by DNA sequencing.²,¹² In this case microdissection and PCR of formalin-fixed tissue was attempted using *Emmonsia* specific primers however no *Emmonsia* specific DNA was identified. This may have been due to the limitations of using formalin-fixed tissue for PCR amplification rather than fresh tissue. Fungal culture was not attempted, but culture of *E. crescens* is reported to be challenging and frequently unsuccessful.²

Recent phylogenetic studies have led to taxonomic revisions of *E. parva* and *E. crescens*. In one study, *E. parva* was shown to cluster in the *Blastomyces* genus (*B. parvus*)⁴, and other members of the *Emmonsia* genus with a yeast tissue form have been reclassified into the *Emergomyces* genus. It has, therefore, been proposed that *Emmonsia crescens* be assigned to the new genus *Adiaspiromyces*.⁴,⁶

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**JPC Diagnosis:** Lung: Pneumonia, interstitial, granulomatous, diffuse, moderate, with numerous extracellular adiaspores.

**JPC Comment:** The contributor has submitted an excellent review of adiaspiromycosis in animal species, from its pathogenesis to the most recent development in classification of this organism.

Lung, rabbit. Adiaspore cell walls also stain strongly with silver stains, although their necessity in this case, like PAS, is questionable. (Gomori methenamine silver, 40X)
Historically, the first description of adiaspiromycosis as a pulmonary infection was first in rabbits by Dr. Chester Wilson Emmons (1900-1985) of the National Institutes of Health. During his tenure at the NIH, he was the first medical mycologist at the NIH as well as the head of the medical mycology section at the National Institute for Allergic and Infectious Disease. Dr. Emmons designated the fungus as *Haplosporium parvum*, but it was reexamined in 1959 by Ciferri and Montemartini, who renamed the organism *Emmonsia parvum*. Interestingly (at least for this particular conference), Dr. C.W. Emmons, during his tenure at NIH, is also credited for identifying the ecological niche for *Cryptococcus neoformans*.

In 1960 Emmons himself added a second species, *E. crescens*, and proposed adiaspiromycosis as a more descriptive name for the disease. The name adiaspiromycosis is derived from Greek - “a” for not or without, “dia” – by or through, and sporo, for seed - ultimately in reference to the fact that the spores to do replicate or disseminate within tissue. The severity of disease is largely the result of inoculum size and the ability of the host to mount an immune response.¹³

The first human case of adiaspiromycosis was reported in 1964, and most cases of this relatively rare fungal infection have been reported to the result of *E. crescens* (which has been reported in over 120 mammalian species as well.) Most cases of *E. parva* have been seen in immunosuppressed hosts. A syndrome of granulomatous conjunctivitis in children in the Amazon basin has been described with *Emmonsia*,¹³ although *Rhinosporidium* could not be completely excluded.

Until recently, *Emmonsia* was limited to two species, *E. crescens* and *E. parvum*. However, a cluster of new *Emmonsia*-like fungi have been isolated in human patients with atypical disseminated mycotic infections behaving more like more traditional dimorphic fungi such as *Blastomyces*, highlighting the close relationship of these two genera. (In fact, *E. parva* is theorized to be closer genetically to *Blastomyces dermatitidis* than to *E. crescens*. Seven new species of *Emmonsia* have been identified since the 1990’s (usually in individual immunosuppressed patients), with only one becoming a named species (*E. pasteuriana*.) Newer species of *Emmonsia* also differ from the historical members of the genus in that rather than exhibiting simple increase in size, they possess the ability to convert to replicative yeasts and disseminate to other tissues.¹³
References:


6. Hughes K, Borman AM. Adiaspiromycosis in a wild European rabbit, and a review of the literature. *J Vet Diagnostic Investig*. 2018; Published online May 2.


