Joint Pathology Center

Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2016-2017

C o n f e r e n c e 19

15 February 2017

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CASE I: 15-0609 (JPC 4067574).

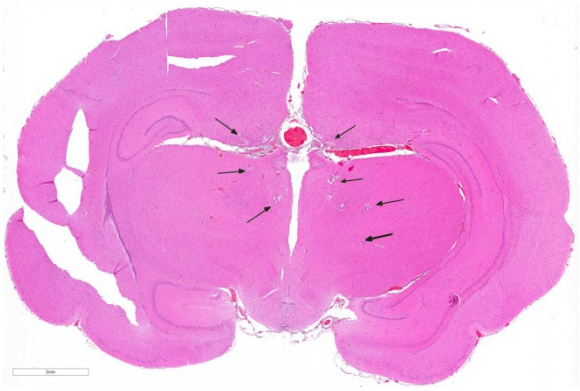
Signalment: Adult, male, eastern gray squirrel, (*Sciurus carolinensis*).

History: Three recuperating eastern gray squirrels were housed in an outdoor cage at a wildlife rehabilitation facility. Shortly after being fed a whole pumpkin, one was found dead, and the other two were ataxic, disoriented, and pawing the air. The pupils were dilated. The attending veterinarian began treatment with fluids, a non-steroidal anti-inflammatory drug (meloxicam), and an anti-convulsant (gabapentin). The neurologic signs did not improve with treatment and time. The squirrels were humanly euthanized, and a necropsy was performed.

Gross Pathology: No gross lesions noted.

Laboratory results: No laboratory analysis performed.

Histopathologic Description: Cerebrum: Multifocally, there is necrosis of both gray and white matter characterized by disruption and loss of neural tissue with replacement by moderate numbers of macrophages with vacuolated cytoplasm (gitter cells), lymphocytes, few plasma cells, and rare eosinophils. Scattered throughout the neural tissue are cross and tangential sections of larval nematodes. The larvae are 50 µm in diameter, have a 5 µm thick cuticle with lateral chords and alae, coelomyarian-polymyarian musculature, a pseudocoelom, and an intestine lined by uninucleate columnar cells with a brush border. Some larvae are associated with areas of necrosis and inflammation and some have no

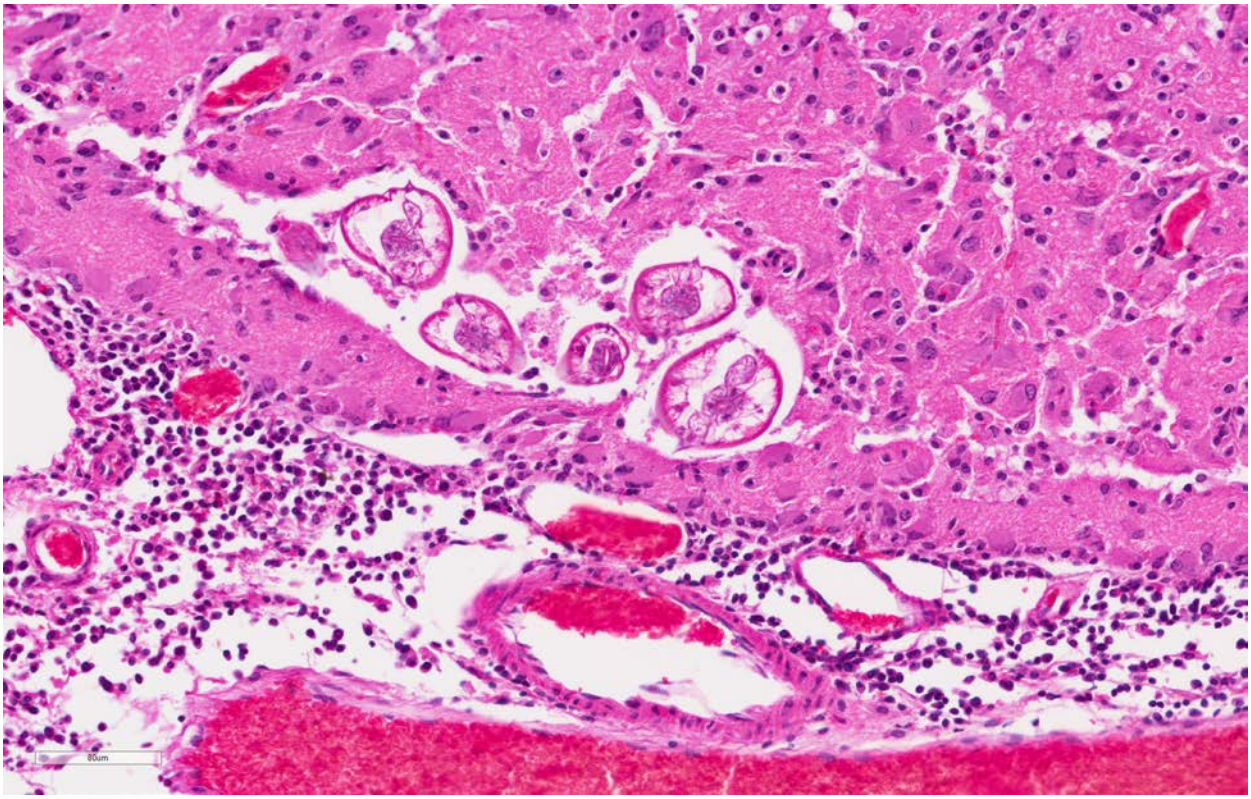


Diencephalon, squirrel. There are multifocal areas of cavitation within the hippocampus and thalamus (arrows). (HE, 5X)

surrounding inflammation but are associated with adjacent vague linear tracks of necrosis and inflammation. Multifocally, throughout the section, there are moderate numbers of lymphocytes with fewer plasma cells and eosinophils expanding perivascular spaces and the meninges. There are moderately increased numbers of glial cells (gliosis) along with spheroids and numerous reactive astrocytes in affected areas.

Contributor's Morphologic Diagnosis: Cerebrum: Meningoencephalitis, necrotizing and granulomatous, multifocal, marked, with perivascular cuffing, gliosis, astrocytosis, spheroids, and numerous larval nematodes.

Contributor's Comment: The initial clinical suspicion was that these squirrels were suffering from a toxin which was associated with the pumpkin. Examination of the enclosure revealed desiccated feces, which when evaluated by fecal floatation revealed the presence of ascarid eggs, consistent with *Baylisascaris procyonis*. Subsequently, histology of the brain provided the definitive diagnosis of neural larval migrans (NLM). *B. procyonis* is a member of the ascarid (roundworms) group of nematodes.[2] The raccoon (*Procyon lotor*) is the definitive host.[3] Dogs can also become infected with *B. procyonis* and shed eggs in feces.[1] Raccoons become infected by consuming *B. procyonis* eggs or intermediate hosts infected with larvae.[1,3] Larvae develop into adults in the raccoon intestine and produce large numbers of eggs which are shed in the feces.[3] Intermediate hosts ingest the eggs in environments contaminated with raccoon feces. In intermediate hosts, the larvae migrate



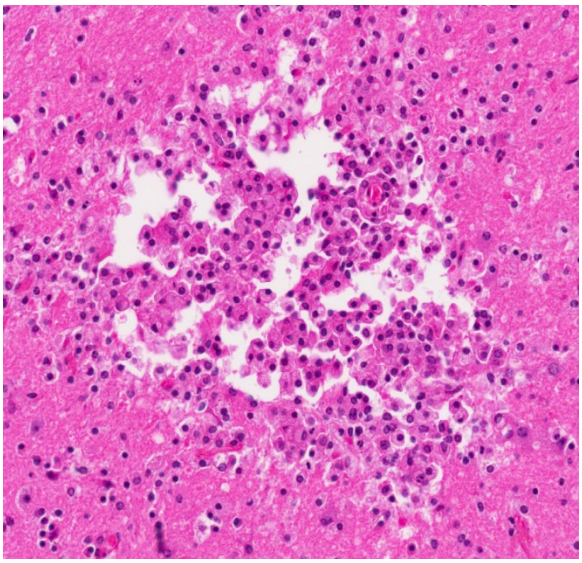
Diencephalon, squirrel. Areas of necrosis occasionally contain cross sections of larval ascarids with prominent lateral alae. Adjacent meninges are expanded by eosinophils and histiocytes. (HE, 228X)

through the body of the host. The third stage larvae of *B. procyonis* can be up to 2 mm in length.[1]

In most tissue, larvae are encapsulated in granulomas, however, in the brain encapsulation is slow to absent.[1] The large larvae migrate through neural tissue causing mechanical damage followed by an inflammatory response.[1,3] In this case, several larvae are present in neural tissue with no surrounding inflammation. Potentially, the pace of the migrating larva was faster than the immune response. Most inflammation present is associated with the migration tracks.

Eosinophils are a typical feature of parasite-induced inflammation, but were less apparent in this case. There may have been some modulation of the immune response in this squirrel due to treatment. In this case, several larvae were present in the brain. However, even low numbers of larvae can result in severe pathology due to their large size and aggressive migration.[1,3] A variety of mammals and birds have been reported as intermediate hosts of *B.* procyonis.[3] *B. procyonis* NLM is also recognized as an important zoonotic disease which predominantly affects children who engage in pica.[1] The eggs of *B. procyonis* can remain viable in the environment for years, even at below freezing temperatures.[3] The hardiness of the eggs and low infectious dose have even lead one investigator to speculate that *B. procyonis* could be utilized as a possible agent of bioterrorism.[5]

JPC Diagnosis: Cerebrum: Encephalitis, necrotizing, granulomatous and eosinophilic, multifocal, moderate with perivascular cuffing, gliosis, and numerous nematode larvae, eastern gray squirrel, *Sciurus carolinensis*.



Diencephalon, squirrel. Foci of necrosis without larval parasites suggest parasite migration tracks. (HE, 300X)

Conference Comment: This case represents an excellent example of the characteristic eosinophilic and granulomatous inflammation with linear necrotic tracts present in neural larval migration of *Baylisascaris procyonis*, a common ascarid nematode parasite found predominantly in the Midwest, Northeast, and West Coastal United States.[7] As mentioned by the contributor, the raccoon is the definitive host and this roundworm parasite typically does not cause clinical disease in this species.[6] The adult nematodes are confined to the small intestine of the definitive host; however, *Baylisascaris procyonis* neural, somatic (visceral), and ocular larval migration are well documented in humans and over 100 wild and domestic animal species, including over 13 species of birds in North America.[1,3,6,7] In this case, the squirrel was likely exposed to raccoon feces from its enclosure and accidentally ingested infective eggs. In addition to raccoons, other species of have been reported to shed *Baylisascaris procyonis* eggs in their feces, including dogs, kinkajous, and skunks.[3,6,7]

Eggs are infective about two weeks after they are excreted by the raccoon host, and may persist in the environment for months to years. After the eggs are ingested by an intermediate host, the larvae hatch, penetrate the intestinal wall, and then enter the portal circulation. After passing into the arterial circulation, the larvae are distributed throughout the body.[5] A small number of larva enter the brain and aggressive larval migration in predominantly the white matter of the brain and spinal cord often leads to rapid debilitation, fulminant neurologic disease, and death of the intermediate host.[4,5] Humans, nonhuman primates, rodents, rabbits, and birds are reported to be the most susceptible to neural larval migrans.[3] Other *Baylisascaris* species include *B. melis* in badgers, *B. columnaris* in skunks, *B. laevis* in woodchucks, *B. schroederi* in pandas, *B. devosi* in the American pine marten, and *B. transfuga* in bears. Any of the above mentioned *Baylisacaris* sp. can cause similar larval migration lesions if infective fecal eggs are ingested by an intermediate or aberrant host.[5,6]

The conference moderator noted that while in this case numerous cross sections of larva are present and readily identified in the neuroparenchyma, most natural cases only have subtle evidence of larval neural migration tracts without identifiable larval cross sections. Additionally, the larva will continue to migrate after the death of the animal, and migration tracts may not always have associated inflammation and necrosis. There are currently no serologic tests commercially available to distinguish active infection from prior exposure.[7] Definitive diagnosis is based on identifying larva in histologic sections, although a presumptive diagnosis is usually made by a combination of history, clinical signs, and serologic testing.[7]

Contributing Institution:
Walter Reed Army Institute of Research
http://www.wrair.army.mil

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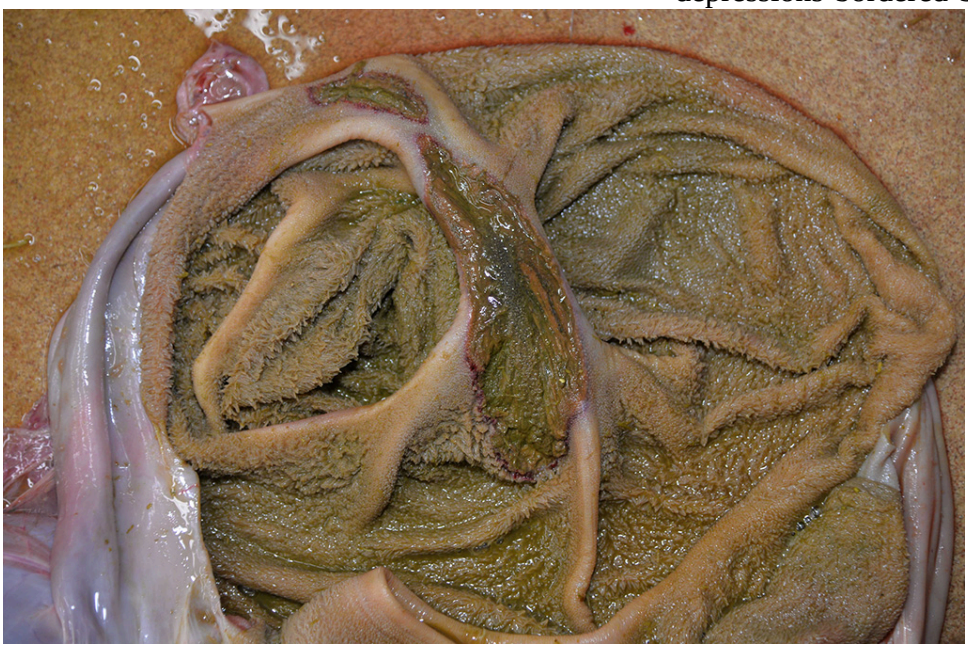
CASE II: WHL16188 (JPC 4084212).

Signalment: Female, Rocky Mountain elk calf (*Cervus elaphus nelsoni*).

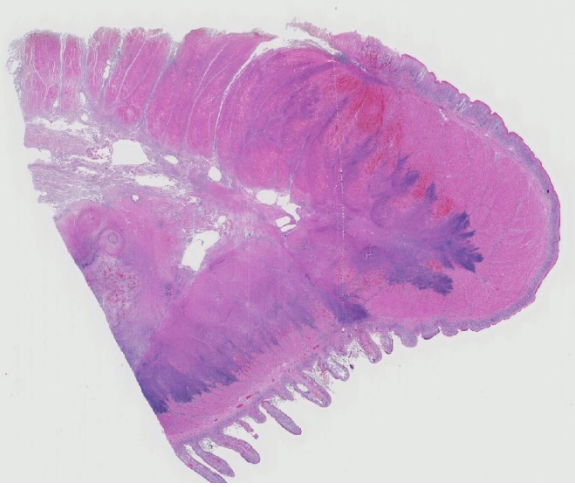
History: Over a three week period, 11 free-ranging Rocky Mountain elk (mostly calves) were found dead at a Colorado ranch in late January.



Rumen, elk calf: The mucosa of the rumen was severely ulcerated, characterized by focal, irregularly round to linear, black, roughened depressions bordered by a hemorrhagic rim. (Photo courtesy of: Colorado State University, Microbiology, Immunology, and Pathology Department, College of Veterinary Medicine and Biomedical Sciences, http://csu-cvmbs.colostate.edu/academics/mip/Pages/default.aspx))

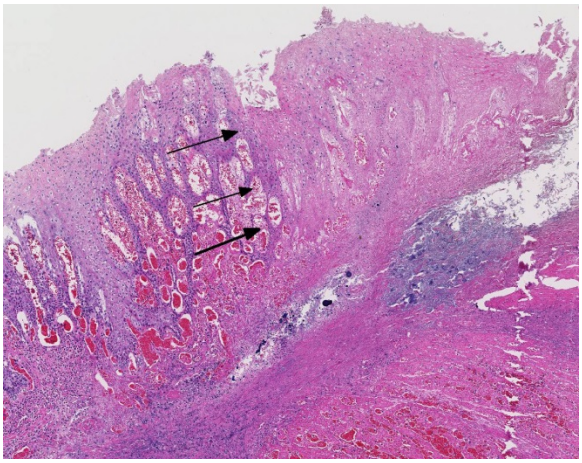
Gross Pathology: Postmortem examination was performed on a female elk calf in poor body condition with mild autolysis. The calf was very thin with minimal fat stores throughout and moderate serous atrophy of fat within the bone marrow (emaciation). Subcutaneous and intramuscular facial edema was prominent in the hind limbs. The abdomen contained scant serous fluid and large, extensive mats of fibrin along serosal surfaces (fibrinous peritonitis). Multifocally, the mucosa of the rumen was severely ulcerated, characterized by focal, irregularly round to linear, black, roughened depressions bordered by a hemorrhagic rim. Ulcerated areas were mural to nearly transmural without perforation. Rumen pillars were most severely affected, and rumen contents were dry. The epicardial surface had moderate multifocal petechial hemorrhages. The ventral margins of the cranioventral lung lobes were dark purple and consolidated.

Laboratory results: *Odocoileus* adenovirus PCR of lung: Positive
Bovine viral diarrhea I&II PCR of lymph node: Negative
Aerobic culture of lung: *Pasteurella multocida*, heavy growth



Esophogeoruminal junction:, elk calf: Large areas of infarction primarily involving the tunica muscularis and serosa are outlined by basophilic cellular debris and hemorrhage. (HE 5X)

Histopathologic Description: Rumen: Affecting approximately 50% of the section is a wedge-shaped region of the rumen obscured by transmural coagulative necrosis with preservation of tissue architecture but loss of cellular detail (infarction). The center of the lesion has regions of eosinophilic cellular and karyorrhectic debris, fibrin deposition, and multifocal mineralization. Necrotic regions are ulcerated and coated by myriad mixed bacteria. They are bordered by extensive hemorrhage, edema, fibrin, infiltration by high numbers of neutrophils, lymphocytes, and plasma cells and mild fibroblastic proliferation (fibroplasia). The walls of vessels throughout the tissue are frequently obscured, either segmentally or diffusely by finely fibrillar, brightly eosinophilic acellular material (fibrinoid necrosis) and moderate to high numbers of neutrophils and lymphocytes admixed with karyorrhectic debris and edema (leukocytoclastic vasculitis). Affected vessels often contain organizing fibrin aggregates and eosinophilic cellular and karyorrhectic debris (vascular thrombosis). Endothelial cells lining affected and unaffected vessels (predominately along the margin of the infarct) are hypertrophic and frequently contain basophilic, smudged, 8-10 μm inclusions that fill the nucleus. The adjacent mucosa and submucosa are infiltrated by moderate to high numbers of neutrophils and lymphocytes admixed with edema. Intact mucosal epithelium also has intracellular edema fluid, occasionally resulting in ballooning epithelial cells.

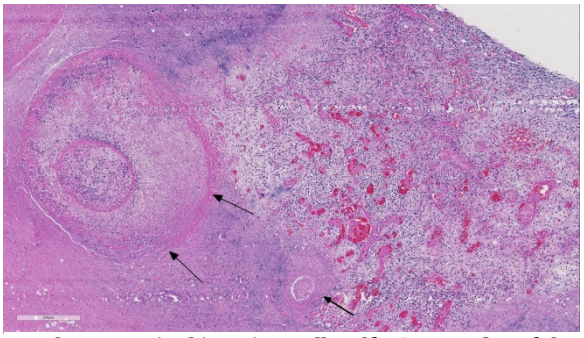


Esophageoruminal junction:, elk calf: At one edge of the section, the necrosis is transmural; necrotic mucosa is has a sharp line of demarcation from adjacent viable mucosa (arrows). (HE, 54X)

Microscopic diagnoses of tissues not submitted:

1. Lungs: Bronchopneumonia, suppurative and necrotizing, subacute, moderate, with mild lymphoid hyperplasia.
2. Lungs: Vasculitis, mild, subacute, multifocal (no viral inclusions detected).
3. Heart: Necrotizing myocarditis, multifocal, mild, acute (no viral inclusions detected).

Contributor's Morphologic Diagnosis: Rumen: Severe necrotizing vasculitis with thrombosis and infarction, subacute to chronic, multifocal, with ulcerative rumenitis and endothelial cell intranuclear viral inclusions consistent with *Odocoileus* adenovirus.



Esophageoruminal junction:, elk calf: At one edge of the section, multiple arterioles show evidence of mural destruction and thrombosis. (HE, 54X)

Contributor's Comment: Adenoviruses are non-enveloped icosahedral viruses that contain double-stranded DNA. A wide range of veterinary species are affected by this class of virus, often resulting in respiratory, gastrointestinal, or systemic disease.[4] In most species, clinical disease is mild, unless the animal is young or immunocompromised.[4] *Odocoileus* adenovirus (OdAdV) was first discovered in mule deer in Northern California in 1993 and was determined to be the causative agent of adenovirus hemorrhagic disease (AHD).[11,12] Since that time, the virus has been found to infect multiple species of deer,[11,13] moose,[8] and more recently, pronghorn and elk (unpublished data). Transmission has been documented via direct contact of cervids and is thought to occur through infected secretions or feces.[13] Studies have shown that OdAdV is most closely related to bovine adenovirus,[5] however, experimental infection studies of domestic cattle with OdAdV have not demonstrated viral replication or disease in this species.[15]

As is true with other adenovirus infections, young animals experience a significantly higher morbidity and mortality rate when compared to adults.[1,9,11] Death can occur as rapidly as five days post-infection, but chronic cases can also occur.[12,13] Acute cases usually have no symptoms or may exhibit ptyalism, diarrhea (with or without blood), weakness, lethargy, anorexia, and edema.[1,9,10] Chronic infections are usually associated with ulcers and abscesses of the mouth and throat, emaciation, and/or sepsis.[1,13]

Disease can be systemic or local.[11] Typical necropsy findings of systemic infections include severe pulmonary edema expanding the interlobular septa with ecchymotic hemorrhages of the lungs and pulmonary artery, and a hemorrhagic enteropathy characterized by hemorrhage within the lumen of the small and large intestine.[12,13] Additionally, multiple ulcerative lesions of the mouth, nasal cavity, mandible, maxilla, and forestomach can be present in systemic disease.[11] Local disease is restricted to the upper alimentary tract (stomatitis, pharyngitis, mandibular/maxillary osteomyelitis, and/or rumenitis).[13] In either form, secondary bacterial infections are common, primarily presenting as abscesses of the oral and nasal cavities.[12-15] Secondary bacterial agents include *Trueperella pyogenes*, *Prevotella* spp., *Fusobacterium necrophorum*, *Streptococcus* spp., *Pasteurella multocida*, and *Peptostreptococcus* spp.[12-15]

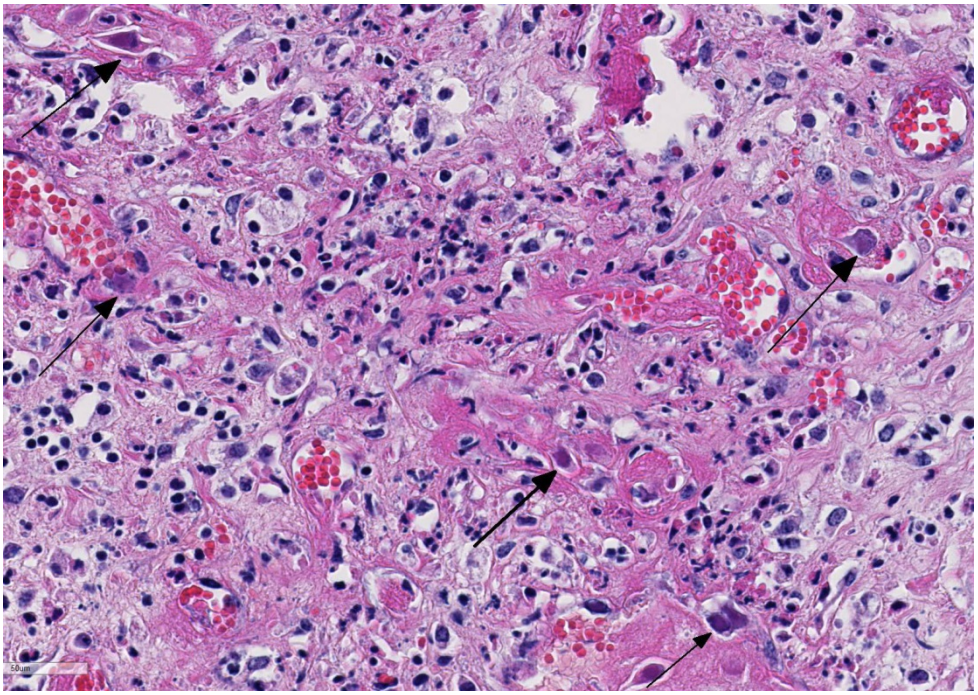
Histologic findings of OdAdV are predominately associated with vascular changes, including endothelial hypertrophy, fibrinoid vascular necrosis, leukocytoclastic vasculitis, and thrombosis, with resulting mucosal ulcerations, tissue infarctions, and extensive edema.[10,11] All types of vessels can be affected and endothelial cell inclusions can be seen in any tissue, with or without significant vascular changes.[12,13] Animals with infection localized to the upper alimentary tract, especially in more chronic infections, can lack endothelial intranuclear inclusions and viral detection methods may be ineffective.[1,11,13] Diagnosis of adenovirus infection in cervids can be

accomplished by histopathology with characteristic endothelial intranuclear inclusions,[11] immunohistochemistry of infected tissue,[12] enzyme-linked immunosorbent assay (ELISA),[6] serum neutralization,[13] virus isolation,[11] immunofluorescence,[11] and PCR (unpublished data).

Differential diagnoses for rumen ulceration include bluetongue (BT) and epizootic hemorrhagic disease of deer (EHD), which are both associated with widespread hemorrhage, edema, necrotizing vasculitis, thrombosis, and ulceration of the alimentary tract.[10] Unlike acute adenovirus infection, inclusions are not detected in tissues with either of these diseases, and additional diagnostic testing may be necessary for definitive diagnosis.[10] In this case, BT and EHD were considered unlikely based on the time of year, since the insect vector (*Culicoides*) is inactive during the winter in Colorado. However, in the warmer season and with chronic cases of OdAdV where inclusions may not be present, all three hemorrhagic diseases (BT, EHD, and AHD) should be considered. Additional differentials for rumen ulceration include mycotic rumenitis and rumen acidosis with Fusobacterium necrophorum infection (necrobacillosis).

In this case, the cause of death was likely multifactorial: malnutrition (emaciation), *Pasteurella multocida* infection, and OdAdV infection. Pasteurellosis is a known cause of mortality in elk calves and can result in rapid septicemia and death.[3] For this animal, it was unclear if the pulmonary vasculitis and necrotizing myocarditis were related to *P. multocida* infection and bacterial sepsis, OdAdV, or both. Unfortunately, the definitive role of adenovirus as a primary pathogen in elk and/or a predisposition for other infectious organisms requires further investigation.

JPC Diagnosis: Rumen: Vasculitis, fibrinonecrotic, multifocal to coalescing, subacute, severe with fibrin thrombi, infarction, ulcerative rumenitis, and endothelial intranuclear viral inclusion bodies, Rocky Mountain elk, *Cervus elaphus nelsoni*.



Esophogeoruminal junction:, elk calf: Endothelium lining necrotic and thrombosed vessels occasionally are swollen by large karyomegalic adenoviral inclusions. (HE, 400X)

Conference Comment: We thank the contributor for providing an outstanding example and superb overview of *Odocoileus*

adenovirus (OdAdV) causing adenovirus hemorrhagic disease (AHD) in the rumen of a Rocky Mountain elk. Participants readily identified necrotizing arteritis within the rumen wall with throm-bosis and large geographical areas of infarcted tissue. These infarcted areas correspond to the large ulcerated areas in the rumen seen grossly in the image provided by the contributor. This case nicely demonstrates frequent large basophilic intranuclear inclusion bodies within reactive and hypertrophic endothelial cells, characteristic for endotheliotropic adeno-virus infection.[2,10]

Conference participants discussed the top two differentials for necrotizing vasculitis causing severe hemorrhagic disease in elk and white-tailed deer. Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHD) of the genus *Orbivirus* and family *Reoviridae*, mentioned by the contributor, are the top two differentials that must be considered in cases of widespread hemorrhage, necrotizing vasculitis, edema, thrombosis, and ulceration of the alimentary tract.[1,2,4,10,11-15] EHD and BTV are both spread by the salivary secretions of the biting *Culicoides* spp. vector. In North America, EHD is the mist significant viral disease in the highly susceptible white-tailed deer, and devastating epizootics have been reported in the United States. Elk are generally less severely affected by BTV and EHD infection. Both BTV and EHD circulate throughout North America and outbreaks often occur simultaneously.[10,11-15] Interestingly, both viruses can be concurrently isolated from the same individual *Culicoides* vector. Conference participants also noted that malignant catarrhal fever caused by ovine herpesvirus-2, a highly pathogenic gammaherpesvirus, can also produce intestinal hemorrhage, pulmonary congestion, edema, and petechiae of the spleen, intestines, heart, and liver with lymphocytic necrotizing vasculitis, and thrombosis in cervids and should also be considered as a potential differential in similar cases.[7]

The conference moderator mentioned that orbivirus infection will not produce the characteristic intranuclear viral inclusion bodies of adenovirus. To further differentiate these two diseases, orbiviral disease outbreaks are usually associated with acute onset of fulminant disease with high morbidity and mortality and viral tropism for the microvasculature endothelial cells. OdAdV typically results in a more chronic onset with targeting of the larger vessels and resulting in larger areas of coagulative necrosis.[1,2,4,5,10-15] Additionally, petechial and/or ecchymotic hemorrhages present at the base of the pulmonary artery has been reported to be highly characteristic and potentially pathognomonic for orbivirus infection in susceptible species. As mentioned by the contributor, diagnosis of adenovirus infection is done by a combination of testing modalities, including visualization of the characteristic endothelial intranuclear inclusions with histopathology, immunohistochemistry (IHC), virus isolation, and polymerase chain reaction (PCR).[11]

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CASE III: X-25548-15 (JPC 4085402).

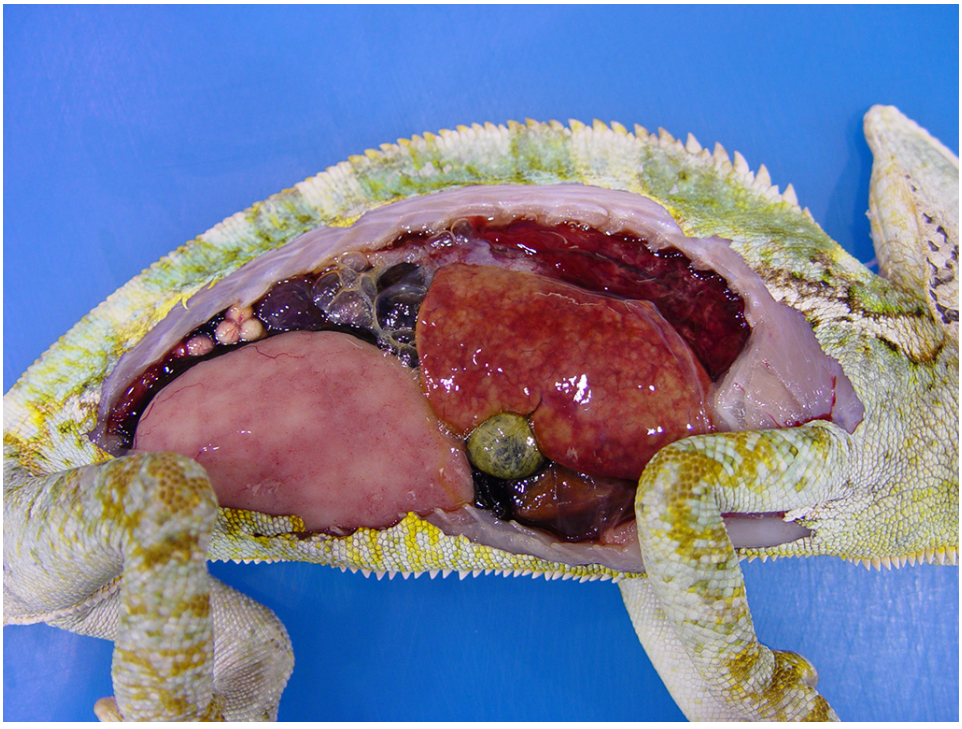
Signalment: Two-year-old, hermaphrodite, veiled chameleon, (*Chamaeleo calyptratus*).

History: Initially the chameleon exhibited open mouth breathing and declining appetite, eventually requiring forced feeding. The patient returned to the clinic 4 weeks later with severe dehydration and obvious weight loss. The chameleon was euthanized at that time.

Gross Pathology: The chameleon was in good nutritional condition with normal muscle mass and moderate coelomic adipose stores. A small amount of clear red-tinged fluid was present in the coelom. Diffuse red discoloration was evident in the expanded lungs and the liver was mottled tan and red with slight rounding of the margins. Ovotestes were present bilaterally. Mucoid content was evident in the lumen of the stomach and the colon; the intestinal content was otherwise scant.

Laboratory results: Sections of lung were submitted for bacterial culture. Primary culture on a sheep blood agar plate after 48 hour aerobic incubation at 37^{o}C resulted in heavy growth of tiny smooth cream-colored colonies. Gram's stain revealed pleomorphic gram-positive bacilli. The bacteria were acid-fast with the Ziehl-Neelsen stain. The isolate was sent to the National Reference Centre for Mycobacteriology (Public Health Agency of Canada). Based on *16s* and *hsp65* gene sequencing, the isolate had 100% sequence identity to *Mycobacterium chelonae* chemovar *niacinogenes.*

Histopathologic Description: Lung: The faveolar septa are expanded with moderate to marked congestion of the vasculature and myriad slender bacilli are present within the lumina of blood vessels; bacteria are both free within the lumina and present within the cytoplasm of macrophages. Hemorrhage, intravascular fibrin thrombi and necrotic cellular debris often accompany the bacterial colonies and scattered small aggregates of epithelioid macrophages are present multifocally in the septa. In areas, there is necrosis of the faveolar epithelium and erythrocytes, proteinaceous fluid and cellular debris are noted with the faveolar spaces.



Lung, chameleon. The lungs are expanded and diffusely red. The liver is enlarged and a mottled tan-red. (Photo courtesy of: Dept of Path/Micro, Atlantic Veterinary College, UPEI, 550 University Avenue, Charlottetown, Prince Edward Island, C1A 4P3. http://www.upei.ca/avc/pathology-and-microbiology)

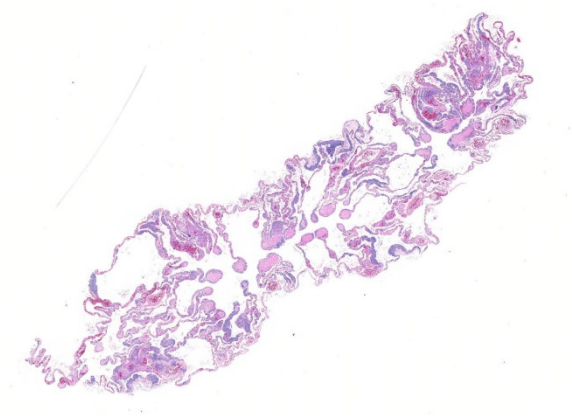
The bacteria are acid-fast with the Ziehl-Neelsen stain.

Similar intravascular bacteria and occasional small aggregates of macrophages containing bacteria are present in most organs (liver, pancreas, kidney, brain, spleen, skeletal muscle, adrenal glands, small intestine and ovotestes).

Contributor's Morphologic Diagnosis: Lung: Necrotizing and histiocytic interstitial pneumonia, diffuse, subacute, with myriad intravascular/intrahistiocytic acid-fast bacilli and intravascular fibrin thrombi

Contributor's Comment: Mycobacteria are ubiquitous in nature and can be isolated from the soil, dust, water and bioaerosols.[1] Reptiles are generally thought to acquire mycobacterial infections via ingestion or through defects / penetrating injury in the skin.[11] In this chameleon, there was a localized area of intestinal ulceration and granulomatous enteritis, suggesting that infection may have been acquired through the intestinal tract.

In reptiles, like most species, mycobacterial infections tend to be chronic with rare acute infections reported.[5] The typical gross lesions are grey-white nodules in multiple organs. Microscopically, early lesions are composed of organized collections of foamy macrophages that with time may become chronic granulomas composed of a mixture of epithelioid macrophages, lymphocytes, plasma cells, and multinucleated giant cells often surrounding a central region of necrosis and occasionally with a surrounding wall of fibrous connective tissue.[11] In this case, there were a few small early granulomas in multiple organs including the brain, lung, liver, kidneys and intestine. More striking, however, was the presence of myriad intravascular bacteria in



Lung, chameleon. Aggregates of gray macrophages multifocally distend the faveolar septa. (HE, 6X)

multiple organs and frequent intravascular fibrin thrombi. These changes are compatible with acute to subacute infection with acute bacteremia and disseminated intravascular coagulation.

Mycobacteria are broadly divided into two groups: *Mycobacterium tuberculosis* complex and non-tuberculous myco-bacteria.[2] While only non-tuberculous mycobacteria have been reported to cause infections in reptiles, several different species have been associated with these infections. These include *M. confluentis, M. chelonae, M. haemophilum, M. hiberniae, M. neoarum, M. confluentis, M. nonchromogenicum, M. marinum, and M. thamnopheos*.[2,5,11] The most common causes of mycobacteriosis in reptiles are reported to be *M. marinum, M. chelonae* and *M. thamnopheos*.[5] The isolate in this case was confirmed to be *M. chelonae* chemovar *niacinogenes.*

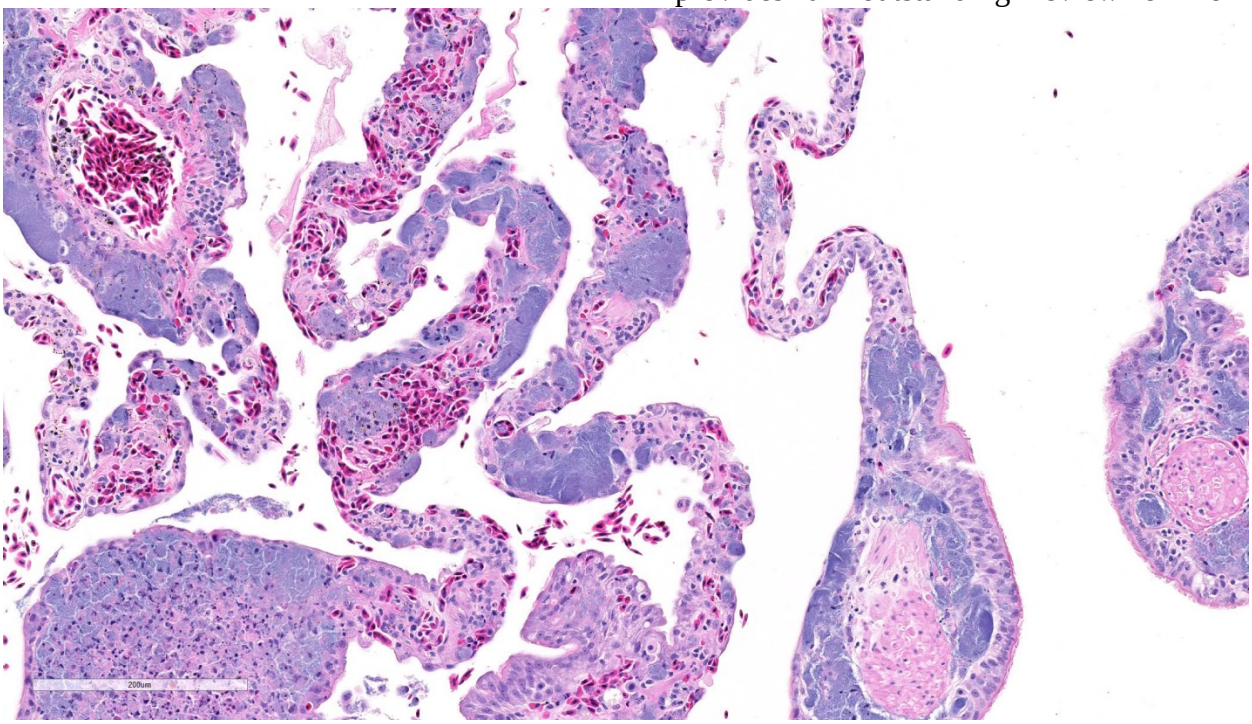
Non-tuberculous mycobacteria are classified into four Runyon groups according to growth rate and pigmentation.[5] Runyon group I mycobacteria are slow growing and form pigment in the light following growth in the dark. Runyon group II organisms are also slow growing bacteria; these bacteria

form pigment in the dark following growth in the light. Runyon group III bacteria are slow growing and do not form pigment in the dark or light. Fast growing non-pigmented mycobacteria are placed in Runyon group IV. These mycobacteria form mature colonies on solid agar within 7 days, while bacteria in Runyon groups I to III take longer periods of time for cultivation.[2] Most mycobacteria which infect reptiles fall into Runyon groups I and IV.[5] *Mycobacterium chelonae* is a rapidly growing mycobacteria belonging to Runyon group IV.[4] Rapidly growing mycobacteria are relatively resistant to standard disinfectants and antibiotic treatment and are increasingly recognized as opportunistic pathogens in humans.[1,2]

in a loggerhead sea turtle[8] and a veiled chameleon.[9] There is a single report of this bacterium causing acute fatal sepsis and disseminated intravascular coagulation in an eastern spiny softshell turtle[8] with lesions very similar to those described in this case. Because of poor response to treatment and the zoonotic potential, euthanasia is often recommended for reptiles with myco-bacterial infections.

JPC Diagnosis: Lung: Pneumonia, histiocytic and necrotizing, multifocal to coalescing, moderate, with numerous intrahistiocytic and intravascular bacilli, veiled chameleon, *Chamaeleo calyptratus*.

Conference Comment: The contributor provides an outstanding review of non-



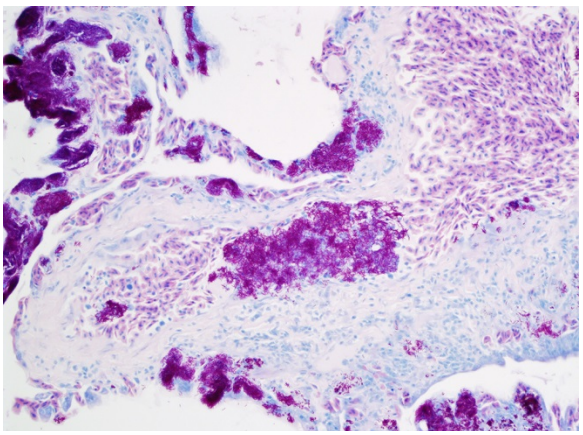
Lung, chameleon. Higher magnification of the faveolar septa. Intracytoplasmic bacilli appear filamentous within macrophage cytoplasm. (HE, 164X)

In reptiles, *M. chelonei* has been reported in association with osteoarthritis in a Kemp's Ridley sea turtle,[4] with stomatitis and subcutaneous granulomas in a boa constrictor,[6] and with disseminated infection

tuberculous *Mycobacteria* in reptiles. *Mycobacteria* spp. are a large genus comprised of over 100 species of obligate pathogenic, potentially pathogenic, and environmental saprophytic bacteria.[6] They

are all morphologically similar and are composed of aerobic, gram-positive, acid-fast, non-spore forming bacilli.[6] Conference participants were impressed by the large numbers of intrahistiocytic and intravascular thin filamentous bacilli that stain slightly basophilic on hematoxylin and eosin stained tissue section. These bacilli are intensely acid-fast positive with Fite-Faraco and Ziehl-Neelsen acid-fast stains, run by the Joint Pathology Center prior to the conference. *Mycobacterium chelonae* infection, confirmed by the contributor as the cause of rapid disseminated disease in this animal, is an opportunistic and potentially zoonotic pathogen that is characterized by rapid growth and high resistance to antibiotics.[1,2]

As mentioned by the contributor, spontaneous non-tuberculous *Mycobacteria* sp., including *M. avium*, *M. chelonae*, *M. szulagai*, *M. fortuitum*, *M. marinum*, *M. hemophilum*, *M. kansasii*, and *M. ulcerans* have been reported to be the classic etiologic agents that cause histiocytic granulomas snakes, turtles, lizards, and crocodiles and should top the list of differential diagnoses for lesions similar to this case.[2,5,10,11] The obligate intracellular bacteria, *Chlamydophila pneumoniae*, can also occasionally infect reptilian species and induce histiocytic granulomas and should be considered as a differential diagnosis. Additionally, relatively recently described "Chlamydia-like" bacteria *Parachlamydia acanthamoebae* and *Simikania negevensis*, have been sporadically reported to form histiocytic granulomas in reptiles as well.[11]



Lung, chameleon: Macrophages contain numerous intracytoplasmic acid-fast bacilli. (Ziehl-Nielson, 400X)

While histiocytic granulomas in reptiles are often induced by intracellular bacteria, such as in this case, heterophilic granulomas in reptiles are caused by extracellular pathogens, including most bacterial and fungal etiologies. Tissue injury can also induce heterophilic granulomas in reptiles.[5] Heterophilic granulomas are characterized by accumulation and degranulation of heterophils leading to a central area of necrosis, stimulating a strong macrophage foreign body-like response. Caseocalcareous nodules, lymphoid infiltration, and peripheral fibrosis, typical of mammalian granulomas, have not been observed in reptilian heterophilic granulomas.[5] Both histiocytic granulomas and heterophilic granulomas can progress to chronic granulomas, characterized by a fibrous connective tissue capsule, lymphocytes and plasma cell infiltration, and a central area of necrosis with a prominent lamellated appearance.[5]

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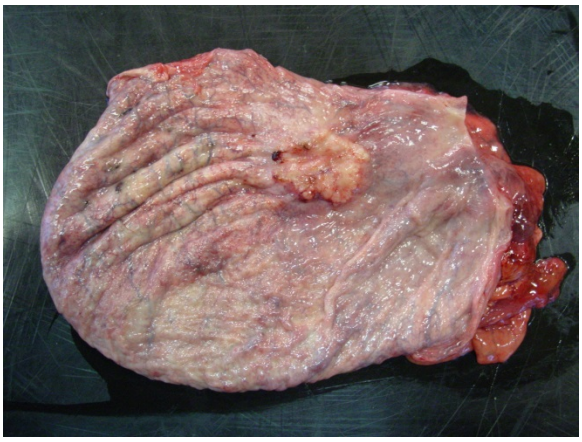
CASE IV: G13060-A786 (JPC 4085315).

Signalment: 25-year-old male California sea lion, (*Zalophus californianus*).

History: This sea lion was born in captivity in a zoo, where it stayed its entire life. Two weeks prior to euthanasia the animal lost 40kg of weight, suffered from ascites and a heart murmur was heard.

Gross Pathology: On post-mortem examination a yellow-white firm nodule was found near the trigone of the bladder, measuring 2 by 3 centimeters and 1 cm in thickness. Similar nodules were found extensively in the liver parenchyma and multifocally in the lung. The lung also



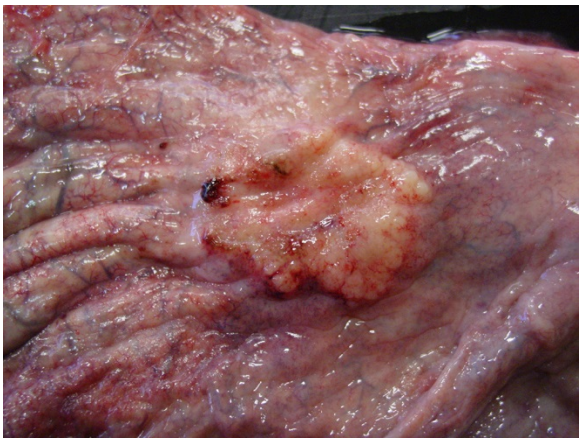
Sea lion, bladder. The bladder wall contains a single yellow-white 2x3cm nodule which is 1cm in thickness. (Photo courtesy of: Dept of Pathology, Bacteriology and Poultry diseases, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke – Belgium, http://www.ugent.be/di/en/departments?ugentid=DI05).

contained large fibrotic interlobular septa. Several lymph nodes were severely enlarged, such as the mesenteric, pulmonary lymph nodes, and one lymph node close to the adrenal gland. The intestines were attached to one another and a large deposition of fibrin together with a large volume (700 ml) of serohemorrhagic fluid (fibrinoid exudative peritonitis).

Laboratory values:

Laboratory results:		Current Californian Sea Lion Reference[2]
IONS		
Sodium	148 meq/L	149-156 meq/L
Potassium	5.08 meq/L	3.7-5.0 meq/L
Phosphate	4.7 mg/dL	1.8-7.8 meq/L
KIDNEY		
Serum urea nitrogen (BUN)	109 mg/dL	14-38 mg/dL
Creatinine	1.47 mg/dL	1.1-2.6 mg/dL
LIVER- BILE DUCTS		
Bile acids	7 µmol/L	
AST (GOT)	41 U/L	12-66 U/L
ALT (GPT)	42 U/L	19-71 U/L
Gamma-GT	143 U/L	22-123 U/L
SERUM PROTEINS		
Total protein	7.2 g/dL	6.1-8.5 g/dL

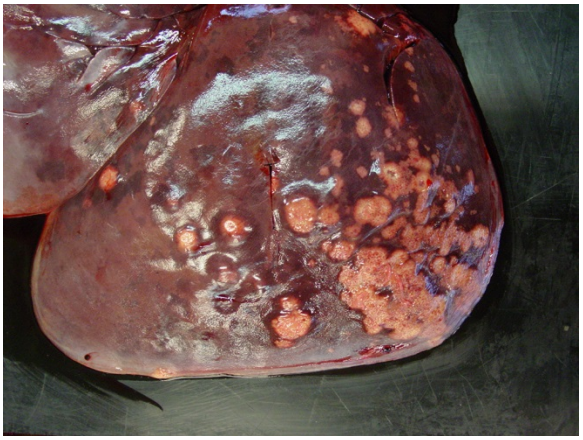
Histopathologic Description: Urinary bladder: Originating from the bladder epithelium and infiltrating in the lamina propria and the muscular layer (*m. detrusor vesicae*), a well-demarcated, densely cellular, multilobular neoplasm is present. In between these lobules lays a dense cellular fibrovascular stroma with large infiltrates of lymphocytes. On some places in the epithelium, multiple groups of disorganized layers of dysplastic cells are visible, which do not breach the basal membrane (carcinoma *in situ*). The neoplastic cells are organized in closely packed smaller lobules and larger nests. The cells have a polygonal appearance, range from 20-40 µm in diameter, contain a moderate amount of light basophilic granular to foamy cytoplasm and have a round to oval, central nucleus with clumped chromatin along the nuclear envelope and 1-2 basophilic nucleoli. Only a few cells have large cytoplasmic vacuoles which pushes the nucleus to the cell periphery which gives the appearance of so-called 'signet cells'. Some cells are multinucleated and there is marked anisokaryosis, anisocytosis, as well as an augmented ratio of nucleus:cytoplasm. Two to five, sometimes bizarre, mitotic figures per HPF are seen. In many lobules of neoplastic cells, central necrosis is present, along with a moderate number of inflammatory cells. Within widened lymphatic vessels and blood vessels, large clumps of neoplastic cells are present showing central necrosis (tumor emboli). Using immunohistochemistry, these neoplastic cells stained positive for low molecular weight cytokeratin and uroplakin and negative for high molecular weight cytokeratin. A PAS-stain on the vacuoles within the 'signet cells' was negative.



Sea lion, bladder. Higher magnification of the neoplasm in the bladder trigone. (Photo courtesy of: Dept of Pathology, Bacteriology and Poultry diseases, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke – Belgium, http://www.ugent.be/di/en/departments?ugentid=DI05)

Various metastases of this neoplasm were found in lymph nodes, liver, lung, adrenal gland, and eye. These metastases stained positive for uroplakin.

Contributor's Morphologic Diagnosis: Nonpapillary and infiltrating transitional cell carcinoma with multiple metastases to various lymph nodes, liver, lung, adrenal gland, and eye.



Sea lion, bladder. Foci of metastasis were present within the haptic parenchyma. (Photo courtesy of: Dept of Pathology, Bacteriology and Poultry diseases, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke – Belgium, http://www.ugent.be/di/en/departments?ugentid=DI05)

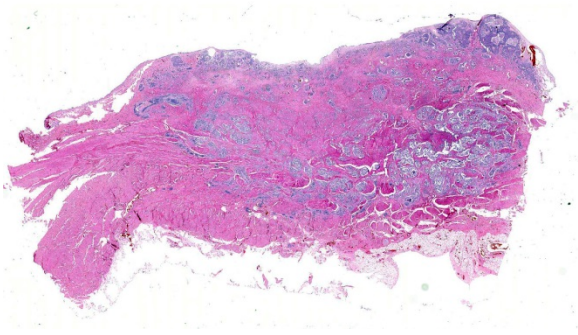
Contributor's Comment: Transitional cell carcinoma (TCC) is the most common tumor of the urinary tract in domestic animals.[9] It is also a common neoplastic lesion in the urogenital tract of the Californian sea lion *(Zalophus Californianus).* In the Californian sea lion, it is a common cause of mortality.[5]

Most TCCs are found in the trigone of the bladder[8] and have a papillary, polypoid, or sessile morphology.[9] Various patterns of TCCs have been described, with the papillary and infiltrating TCC as the most common type and the nonpapillary, infiltrating type as the second most common.[9] Some TCCs may originate in the ureter or urethra and might be more difficult to diagnose.[5]

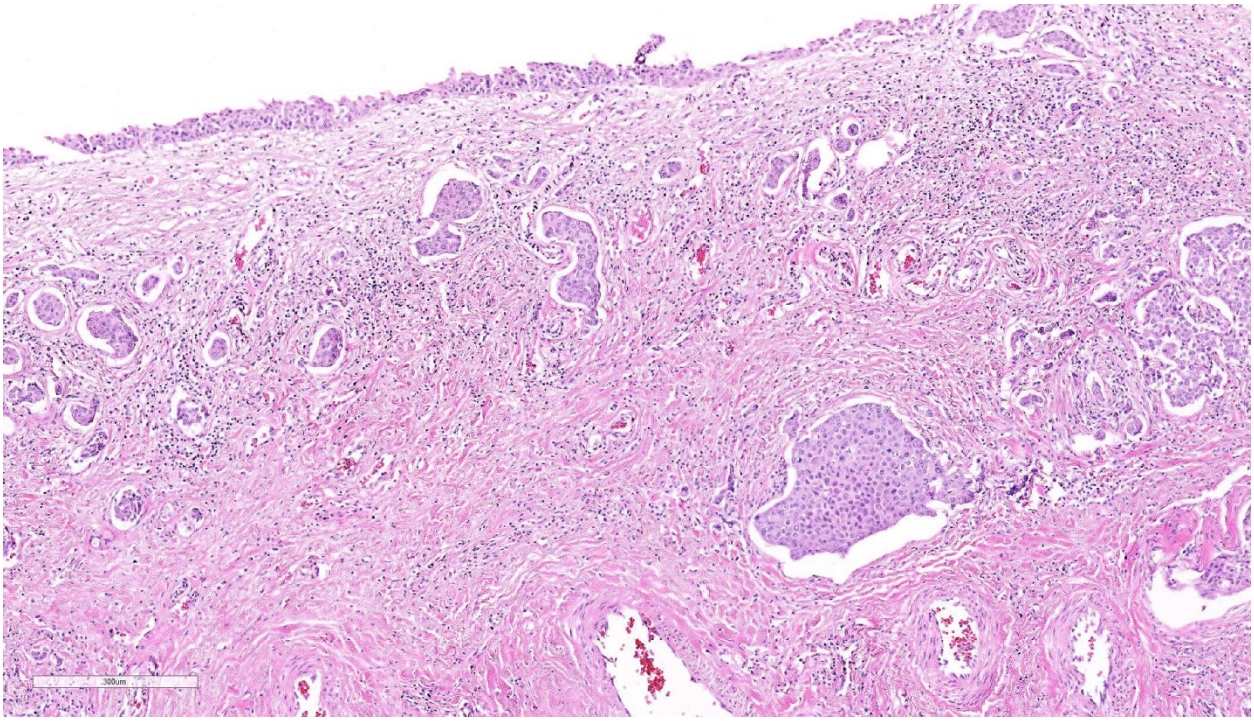
Histologically, nonpapillary infiltrating TCCs show a thickened bladder wall[9] due to neoplastic growth of the urinary epithelium. Neoplastic cells are organized in small nests and form a multilobular pattern.[5] Cyst-like structures may be visible, containing necrotic or keratinized debris may be seen.[5] Neoplastic cells may show cystic degeneration and contain large cytoplasmic vacuoles, pushing the nucleus to the periphery creating a characteristic 'signet ring morphology. The large polygonal to round cells have distinct cell borders[10], pale eosinophilic to amphophilic cytoplasm[5] and contain enlarged pleomorphic to polygonal and vesicular nuclei, with prominent nucleoli[9] and clumped to reticular chromatin.[3] Syncytial cells, atypical nuclei, mitotic figures are abundant.[9] There might be variable desmoplasia and various amount of lymphoid inflammation. Vascular and lymphatic vessel invasion is often seen.[9]

The cause of TCC remains undetermined[6], but is likely multifactorial.[3] Environmental contaminants, such as polycyclic aromatic hydrocarbons like benzopyrine have been suggested to play a role.[5] Lipscomb et al. found intranuclear inclusion bodies in genital carcinoma and isolated a herpes virus (rhadinovirus) and an Epstein-Barr virus using PCR.[6,7] Polychlorinated biphenyls could also contribute to the occurrence this neoplasm.[10]

TCCs metastasize in about 50% of the cases in domestic animals; first affecting regional lymph nodes before spreading to other organs.[9] Metastases of TCC in Californian sea lions is also common, as in the current case[3]. The nonpapillary infiltrating type is the most likely to metastasize.[9] Bilateral hydronephrosis due to obstruction by the



Sea lion, bladder. Nests of neoplastic cells extend downward from the ulcerated bladder mucosa through the full thickness of the urinary bladder. (HE, 5X)

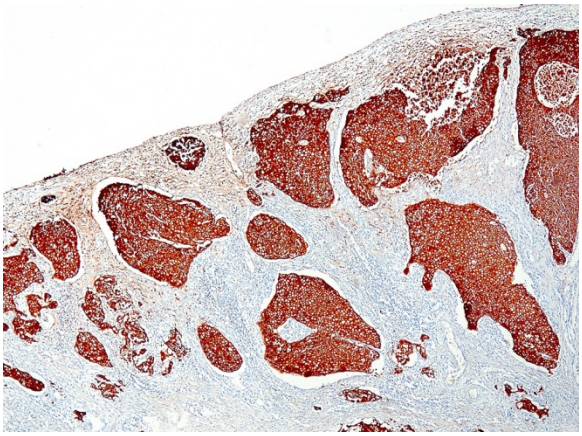


Nests of neoplastic cells are present within the submucosa. There is mild lymphocytic inflammation separating infiltrative nests. (HE, 140X)

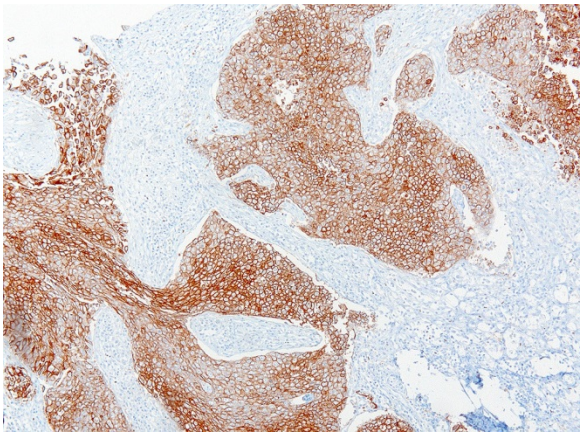
neoplasm in either ureter or urethra is a possible associated lesion.[9]

JPC Diagnosis: Urinary bladder: Carcinoma, metastatic, California sea lion, *Zalophus californianus*.

Conference Comment: This case generated spirited discussion among conference participants regarding the tissue of origin of the numerous small islands and nests of neoplastic cells in the muscular layers of this section of urinary bladder. Most participants agreed with the contributor and favored the diagnosis of urothelial carcinoma (also known as transitional cell carcinoma); however, others preferred the diagnosis of metastatic carcinoma not otherwise specified, because the neoplastic cells generally do not associate with the overlying transitional epithelium, rather forming distinctive intra-epithelial neoplasms (IEN) and distinct islands within the muscular layers of the urinary bladder. IEN has been previously associated with genital carcinoma of California sea lions (CSL) described in similar lesions in the lower urinary tract (vagina, cervix, uterus, prepuce, penis, and urethra).[3,6,7] Interestingly, lesions of the urinary bladder had not been described in previous cases of genital carcinomas in CSL.[6,7] Unfortunately, in this case, most of the hematoxylin and eosin (HE) stained tissue sections provided to participants contained little to no overlying epithelium,



Sea lion, bladder. Neoplastic cells exhibit strong intracytoplasmic staining for cytokeratin. (anti-CK, 100X) (Photo courtesy of: Dept of Pathology, Bacteriology and Poultry diseases, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke – Belgium, http://www.ugent.be/di/en/departments?ugentid=DI05)

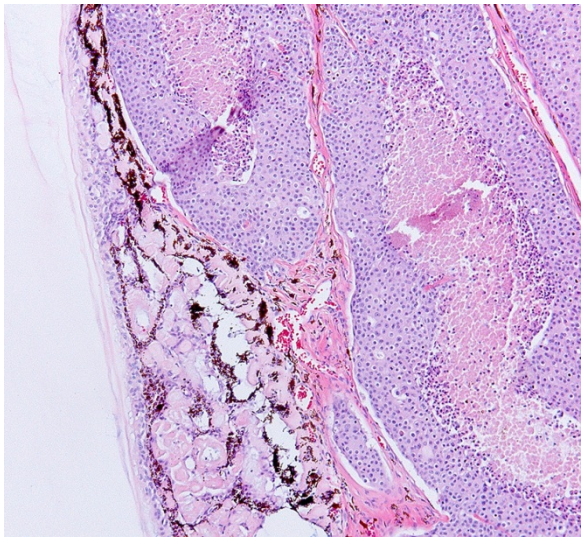


Sea lion, bladder. Neoplastic cells exhibit strong intracytoplasmic staining for uroplakin. (anti-uroplakin, 200X) (Photo courtesy of: Dept of Pathology, Bacteriology and Poultry diseases, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke – Belgium, http://www.ugent.be/di/en/departments?ugentid=DI05)

so it was difficult to accurately assess the origin of the neoplasm. We thank the contributor for proving excellent quality images of strong and highly specific membranous immunoreactivity of the neoplastic cells for uroplakin-III in both the urinary bladder and eye metastasis.[11] Prior to the conference, the Joint Pathology Center also ran uroplakin-III and pancytokeratin and confirmed strong immunoreactivity of the neoplastic cells for both stains.

The mammalian urothelium is composed of 6–10 layers of cells that includes superficial, intermediate, and basal layers.[2,12] The superficial layer, commonly known as the umbrella cell layer, contains a specialized plasma membrane that forms rigid plaques covering the apical surface of the urothelium. Uroplakin is one of the major protein components of the umbrella cells in the superficial layer and is highly conserved through most, if not all, mammalian species. The immunohistochemical stain, uroplakin-III, is marker for urothelial cells of the renal pelvis, ureter, bladder and urethra and has been used to detect transitional cell neoplasms in humans, dogs, cattle, and laboratory rodents. Loss of uroplakin-III expression in bladder cancers has been associated with malignant, invasive and anaplastic urothelial carcinomas in humans and dogs.[2,12] Uroplakin-III is generally considered to be specific for urothelial carcinomas; however, positive immuno-reactivity for uroplakin-III has also been reported in prostatic carcinoma in humans and dogs.[2,12] Positive immunostaining for uroplakin-III provides strong evidence for urothelial origin of the neoplasm in this case; however, further study may be needed to determine the specificity of uroplakin-III in the genital and urinary tract in California sea lions.

Invasive and metastatic genital and urinary tract carcinomas are commonly diagnosed in stranded California sea lions. As mentioned by the contributor, these neoplasms have been associated with a number of predisposing causes, including otariine gamma herpesvirus[6,7] infection and exposure to polychlorinated biphenyl water



Sea lion, bladder. Metastasic cells are present within the ciiary body. (Photo courtesy of: Dept of Pathology, Bacteriology and Poultry diseases, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke – Belgium, http://www.ugent.be/di/en/departments?ugentid=DI05)

pollutants.[3,10] Additionally, there is a reported association between development of urogenital carcinoma in sea lions with genetic homogenicity at the Pv11 microsatellite locus for the heparanase 2 gene (*HPSE2*).[1] Homogenicity at this site is also strongly correlated with decreased overall fitness within the stranded California sea lion population and may be secondary to inbreeding.[1] Conference participants did not note eosinophilic intranuclear inclusion bodies characteristic of herpesvirus infection in this tissue section.

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