Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

C o n f e r e n c e 15

27 January 2016

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National Zoological Park

CASE I: 33177-A (JPC 4002480).

Signalment: 2nd year adult, intact male, red cardinal (*Cardinalis cardinalis*).

History: This wild-caught cardinal presented with a large crusted mass within the skin of the cloacal region and poor body condition. Euthanasia was elected and a necropsy was performed by the referring veterinarian. Formalin-fixed organ/tissue samples were subsequently submitted for histological examination.

Gross Pathology: Focally within the skin and subcutis of the dorsal cloacal region, there is a large crusted mass composed of multiple small (up to approximately 6 mm in diameter), individual, cystic structures containing a soft brownish, amorphous material. Each cyst contains one to two, light grey to tan and roughly ovoid trematode parasites with a concave ventral surface. Each fluke measures 4-6mm long x 4-6mm diameter and is characterized by prominent vitelline glands and an extensively convoluted, white to tan uterus. The mass is focally contiguous with the overlying skin surface via a single, pinpoint pore. No other gross lesions were identified.

Cloaca, cardinal. The skin and subcutis of the dorsal cloacal region contains a large mass of individual cystic structures. (Photo courtesy of: University of Minnesota, Veterinary Diagnostic Laboratory, 1333 Gortner Ave, St Paul, MN, 55108 http://www.vdl.umn.edu/

Laboratory Results:

Parasite identification: Trematode parasites extracted from the dermal pseudocysts were identified as *Collyriclum faba*.

Histopathologic Description: Skin and subcutis (dorsal cloacal region): The dermis and subcutis are expanded by a focal cluster of 3-6mm diameter, spherical to ovoid pseudocysts individually separated and surrounded by thick bands of compressed dermal fibrous connective tissue. The pseudocysts are enclosed by a variably thick (2-40um diameter) collagenous capsule and contain one to two, 3-5mm diameter (in cross section) adult trematodes. These trematodes are characterized by the following features: a 5-7um thick pale eosinophilic cuticle containing numerous brightly eosinophilic spines in pairs or small groups; an anterior sucker and pharynx; prominent vitellaria; a coiled uterus containing large numbers of 20um x 10um, ovoid, brown-shelled eggs (containing miracidia); testes containing numerous basophilic spermatozoa and ovaries. The surrounding thickened dermal and subcuticular connective tissue contains mild to moderate, multifocal to coalescing infiltrates of heterophils, macrophages and lesser numbers of lymphocytes and plasma cells. Occasionally scattered within these regions, there are dense, multifocal infiltrates of macrophages (including multinucleated macrophages) surrounding small clusters of trematode eggs. Overlying the pseudocyst cluster, the skin is multifocally ulcerated and covered by a thick serocellular crust composed of large amounts of eosinophilic and basophilic cellular and keratinaceous debris with entrapped viable and degenerate heterophils, small colonies of basophilic coccoid bacteria and small numbers of trematode eggs.

Cloaca, cardinal. Cystic structures are up to 6mm in diameter. (Photo courtesy of: University of Minnesota, Veterinary Diagnostic Laboratory, 1333 Gortner Ave, St Paul, MN, 55108 http://www.vdl.umn.edu/)

Blood vessels throughout the section contain large numbers of blood cells parasitized by protozoal organisms (e.g. *Leukocytozoon* sp. and/or *Hemoproteus* sp.). In one field, a skeletal muscle myofiber is expanded by a large sarcocyst containing numerous bradyzoites.

Contributor's Morphologic Diagnosis:

1) Skin and subcutis (dorsal cloacal region): Parasitic pseudocysts, multifocal, with ulcerative and granulocytic dermatitis and intralesional mature adult flukes and fluke ova, etiology consistent with *Collyriclum faba*
2) Skeletal muscle: Sarcocystosis, mild
3) Protozoal parasitemia, marked

Contributor's Comment: *Collyriclum faba* is a digenetic trematode which uncommonly infects passeriform and galliform birds of Eurasia and North and South America.[1,5,7] The adult trematodes are uniquely located within the peri-cloacal skin and subcutis and less commonly, the skin of the ventrum, limbs, head and neck.[7] The parasites form discrete encapsulated pseudocysts containing paired, juxtaposed adult forms. Macroscopically, compared with most

Cloaca, cardinal. Cross section of the cloacal mass, showing several closely apposed pseudocysts. (HE, 4X).

trematodes, *Collyriclum faba* is dorsally convex and ventrally flattened with a dorsal rather than ventral sub-terminal oral sucker.[1] The histological characteristics of the trematodes include: a thick pale eosinophilic, poorly muscular cuticle containing numerous brightly eosinophilic spines in pairs or small groups; a dorsal sucker; bifurcated intestinal crura; prominent vitellaria; a coiled uterus containing operculated brown-shelled eggs; reproductive organs.[1,7]

The life cycle of this parasite is incompletely understood, however, birds are thought to become infected following ingestion of metacercariae in the putative snail or dragonfly intermediate hosts.[3,7] Fluke eggs are thought to be released from a pore in the dermal pseudocysts of the avian host upon immersion in water where they then infect the intermediate hosts.[3,6] This phenomenon has been demonstrated by rubbing wet gauze over the pseudocysts in live avian patients.[3] *Collyriclum faba* is not usually associated with clinically significant disease, though it has been associated with cloacal obstruction and emaciation in some cases.[3]

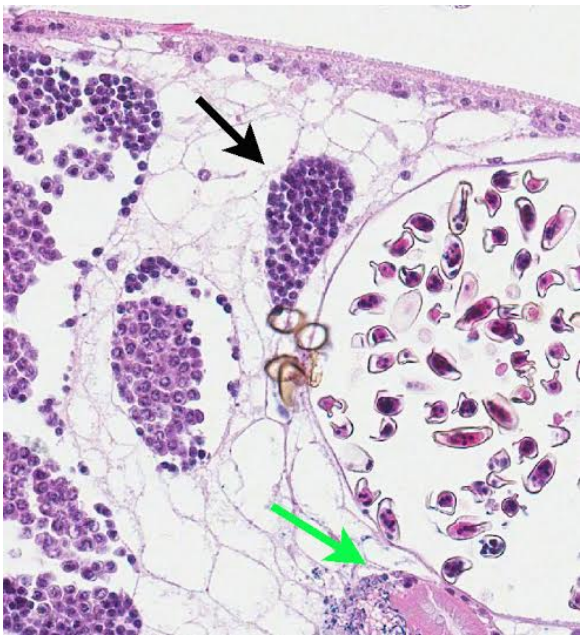
JPC Diagnosis: 1. Glabrous skin: Multiple trematode pseudocysts with mild heterophilic and granulomatous dermatitis.

2. Skeletal muscle: Sarcocysts, multiple (some sections).

3. Circulating erythrocytes: Intracytoplasmic protozoa.

Conference Comment: The prevalence of infection and specificity of *C. faba* for certain bird species is thought to be related to the distribution of intermediate hosts and diet of the definitive host. *C. faba* has been identified in both wild avian species as well as domestic poultry.[3] The first intermediate host, a small freshwater snail, has a relatively limited distribution, while the second intermediate host, various species of mayfly, as well as the definitive avian hosts, have a larger range. As mentioned above by the contributor, adults of *C. faba* form pseudocysts, which are located in the subcutis and occur in pairs. Birds will most often harbor up to four cysts, and as mentioned above are not generally associated with clinical disease; however, heavily parasitized birds may be anemic, emaciated, have problems defecating and eventually die. The parasitism may occur in combination with viral disease such as West Nile virus, in which case severity of infection is increased.[4] Increased numbers of

Cloaca, cardinal. Each pseudocyst contains cross sections of two flukes (arrows). (HE, 10X)

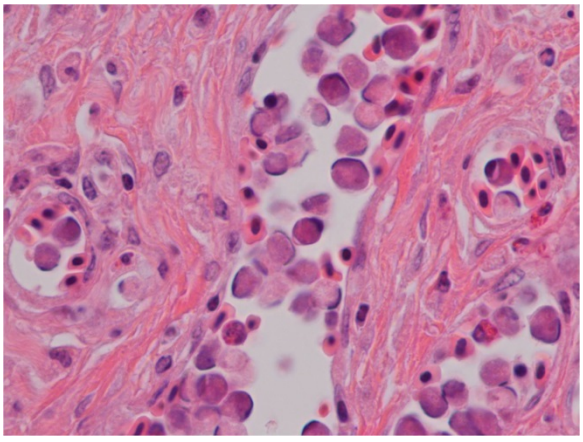


Cloaca, cardinal. The flukes have a thin serrated cuticle, a large uterus containing brown-shelled eggs, cross sections of a testis containing spermatocytes (black arrows) and ceca (green arrow) (HE, 120X).

cysts can also be associated with increased morbidity and mortality.[5]

There is slide variability with few sarcocysts being present in skeletal muscle in some sections. *Sarcocystis* spp. protozoan parasites are common incidental findings in various mammalian and avian species. Infective sporocysts are shed in the feces of the definitive host, which are ingested by the intermediate host, where asexual reproduction occurs resulting in the formation of cysts in the skeletal muscle. One common species which infects birds is *S. falcatula*; the Virginia opossum is the definitive host and many different species of birds serve as intermediate hosts. The birds ingest infective sporocysts in the opossum's feces, or by ingesting transport hosts such as cockroaches. In addition to skeletal muscle, sarcocysts may also be found in cardiac muscle and the central nervous system. The definitive host is infected by ingesting a bird which contains sarcocysts. The cysts are digested and zoites invade the intestinal epithelium of the definitive host, developing into gamonts. Fertilization occurs, resulting in the formation of oocysts, which sporulate and are shed as the infective form in the feces. The intermediate host ingests feces with sporocysts; once the sporocysts are ingested, the sporozoites are released in the intestine, where they migrate to blood vessels, eventually developing into meronts. Merozoites are eventually liberated from the meronts and enter circulation, resulting in their migration to cardiac, skeletal muscle and nervous tissue where they develop into sarcocysts, containing metrocytes that produce bradyzoites, which are infective to the definitive host.[2]

The conference histologic description was very similar to the contributor's thorough description above. Multiple sections of a small tubular structure, lined by cuboidal to columnar epithelium and surrounded by smooth muscle, are present at the periphery of the section and these structures were tentatively identified as the vas deferens. The excellent gross images submitted with this case were also discussed. Conference participants commented on the nodular and ulcerative nature of the lesion as well as the



Cloaca, cardinal. The vast majority of leukocytes in the peripheral blood contain a single glassy intracytoplasmic hemogregarine schizont which peripheralizes the nucleus. (HE, 600X)

feather loss and poor body condition, indicated by keel prominence and the absence of subcutaneous fat. The dark brown foci seen grossly overlying the pseudocysts were postulated to be foci of ulceration secondary to rupture and release of eggs. Although difficult to resolve even at high power, the egg shells have small hyalinized spines. Conference participants discussed the different hemoprotozoan genera including *Plasmodium*, *Haemoproteus* and *Leucocytozoon* but agreed it was not possible to definitively determine which organism is present in this case.

Contributing Institution:
University of Minnesota
http://www.vdl.umn.edu/

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CASE II: 15-0608 (JPC 4066922).

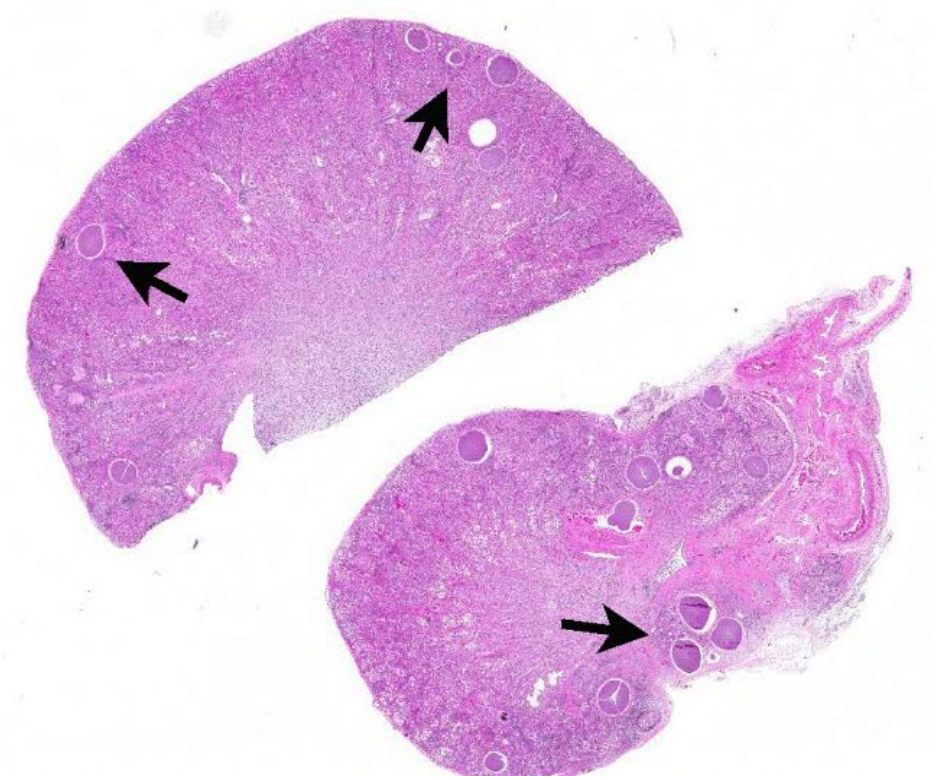
Signalment: Adult female opossum (*Didelphis virginiana*).

History: A Good Samaritan found this opossum on a road side and transported it to a wildlife rehabilitation center. The attending veterinarian examined the opossum and found the following: emaciation and dehydration; an abrasion on the right dorsum; a scab with protruding bone at the tail tip; a bruised and swollen left elbow; and numerous small white nodules in the skin of the muzzle, ears and anus. Radiographs revealed a fracture of the left ulna. The opossum died while under isoflurane anesthesia and the attending veterinarian performed a necropsy.

Gross Pathology: There were numerous 1mm in diameter white nodules in the kidney, adrenal glands, spleen, heart, ovary, and haired skin and subcutis.

Laboratory Results: NA

Histopathologic Description: Kidney: Multifocally, throughout the cortex, there are many round protozoal cysts up to 850-1000 µm in diameter that compress adjacent nephrons. The cysts are composed of densely packed crescentic bradyzoites that are 4 µm long surrounded by a 3-8 µm thick rim of host cell cytoplasm which is enclosed



Kidney, opossum. There are multiple round apicomplexan cysts within the renal cortex. (HE, 5X).

in 20-40 µm thick, hyaline capsule. Multifocally, extending from the cortex to the medulla, the interstitium is disrupted and expanded by aggregates of moderate numbers of lymphocytes admixed with fewer plasma cells, and neutrophils. Multifocally, cortical tubules are dilated with attenuated epithelium and contain proteinaceous fluid admixed with few sloughed cells and cellular debris. There are few tubules lined with swollen vacuolated epithelial cells (degeneration). There is also loss of tubules with replacement by fibrous connective tissue. Multifocally, few glomerular tufts are expanded by an eosinophilic homogeneous material.

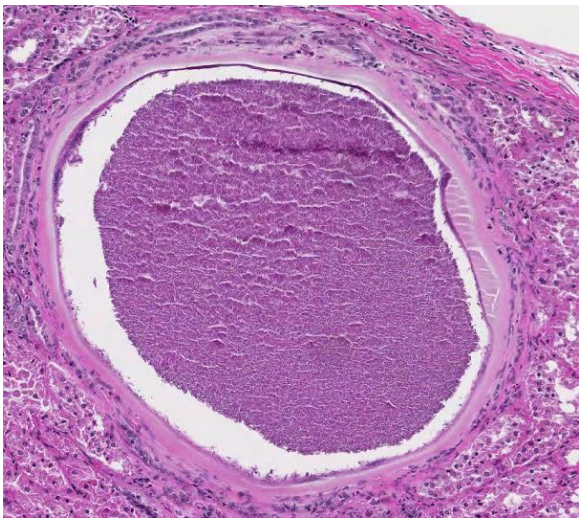
Contributor's Morphologic Diagnosis:

Kidney: Protozoal cysts, numerous, etiology consistent with *Besnoitia* spp**.**
Kidney: Nephritis, interstitial, lymphoplasmacytic and neutrophilic, chronic, multifocal, marked, with membranous glomerulonephritis, and interstitial fibrosis.

Not submitted:
Haired skin, ovary, adrenal gland, spleen, and heart: Protozoal cysts, numerous, etiology consistent with *Besnoitia* spp.

Lung:
Bronchopneumonia, granulomatous, multifocal, marked, with adult nematodes, larvae and eggs, etiology consistent with *Capillaria spp.* and *Didelphostrongylus spp.*
Spleen: Amyloidosis, multifocal, marked.

Contributor's Comment: The role that the *Besnoitia* parasitism played in the pathology of the kidney is not clear. There are *Besnoitia* cysts surrounded by inflammatory cells and some without, and there are aggregates of inflammatory cells not associated with cysts. The cysts are predominantly intact. *Besnoitia* is not typically associated with clinical disease in the opossum, though cases with morbidity have been reported.[1,6] While the cause of the chronic interstitial nephritis is not apparent in the submitted sections, this opossum was likely stressed, immunosuppressed and experiencing chronic antigenic stimulation. The open tail wound, the fractured ulna, the skin abrasion, the bronchopneumonia, and the *Besnoita* all likely contributed to the chronic antigenic



Kidney, opossum. Apicomplexan cysts measure up to 40um with a thick hyaline capsule and large numbers of zoites. (HE, 40X)

stimulation which resulted in the glomerular changes in the kidney and amyloid deposition in the spleen.

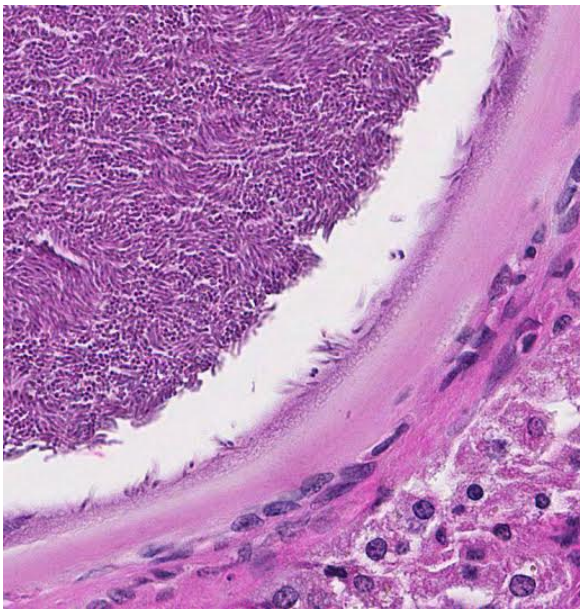
Besnoitia is a protozoal parasite in the phylum *Apicomplexa*.[3] *Besnoitia* spp. require two hosts (heteroxenous life cycle).[1,6] The domestic cat has been demonstrated to act as a definitive host for *Besnoitia darlingi* (species associated with opossums), in which infectious oocysts develop and are shed.[7] Opossums and other species act as intermediate hosts, in which oocysts develop into tissue cysts.[2,7] Opossums become infected by ingestion of oocysts from cats or tissue cysts by consumption of infected tissue from other intermediate hosts.[2,6,7]

JPC Diagnosis: 1. Kidney: Tubulo-interstitial nephritis, neutrophilic and histiocytic, chronic diffuse, marked with intratubular leptospires.

2. Kidney: Amyloidosis, interstitial, multifocal, mild.

3. Kidney: Apicomplexan cysts, multiple.

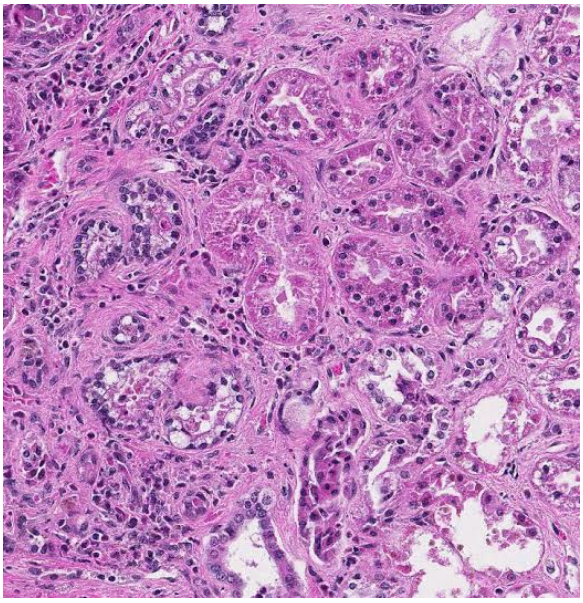
Conference Comment:
Besnoitia spp. protozoan parasites infect a wide variety of both wild and domestic mammalian species. The cysts are most commonly seen in visceral organs, skin, and skeletal muscle. As mentioned above, *Besnoitia* spp. infection in the opossum generally does not produce clinical disease; however, cases with significant lesions and clinical disease have been reported. Clinical disease is generally associated with immunosuppression, stress, and young age. The precise reason some opossums are afflicted by more severe disease is unclear, but other comorbidities may also play a role. In reported clinical cases, protozoal cysts presenting as white nodules at multiple locations in both eyes and nodules throughout the skin were predominant findings; varying degrees and types of ocular pathology were also observed. White nodules were also reported in the oral cavity, lungs, skeletal muscle and heart, specifically in the myocardium where protozoal cysts, foci of mineralization and inflammation were noted. Mineralized hyperechoic



Kidney, opossum. Higher magnification of the apicomplexan cyst. (HE, 100X).

nodules were also reported in the kidneys as well as varying degrees of interstitial nephritis and tubular changes. Foci of mineralization were also described in various other tissues.[1] Another study reported protozoal cysts in other organs such as the liver, spleen, stomach and lung. In that same study, debilitated adult female opossums were the most affected subgroup, the ear was the most frequently reported site for protozoal cysts, and the majority of infections were seen in the summer season.[2]

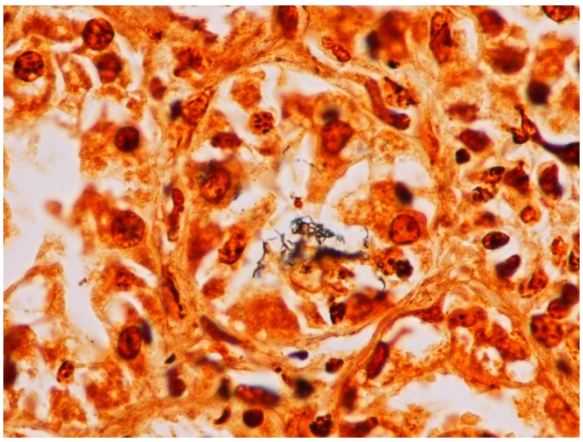
Histologically, the cysts consist of a single enlarged host cell, within which abundant crescent-shaped bradyzoites are packed into a parasitophorous vacuole, which fills the enlarged host cell. The host cell cytoplasm is present as a thin rim at the margin of the enlarged cell. Elongated host cell nuclei may be seen as an inner cyst membrane. The outer layer of the cyst, which forms the capsule, is seen as a variably thick, hyalinized layer of collagen fibers[2] which stains blue with Masson's trichrome stain.[5]



Kidney, opossum. Chronic changes in the remainder of the kidney include marked interstitial fibrosis with tubular degeneration and atrophy. (HE, 260X).

Mineralization of cysts may be seen as well as varying degrees of host inflammatory reaction.[2] *Besnoitia* spp. infections are well documented in other mammalian species, including cattle which may become infected with *B. besnoiti*. Recently, a new cyst nomenclature has been proposed for that species, due to historical inconsistencies in cyst descriptions consisting of the following: Hypertrophied host cell, enlarge nuclei, intracytoplasmic parasitophorous vacuole which contains bradyzoites, an inner cyst wall that may be vacuolated and an outer cyst wall in better developed cysts., and includes tissue cysts This entire structure is referred to as a tissue cyst. In cattle, the condition occurs in acute, subacute and chronic stages. The acute stage is associated with endothelial infections and resultant vascular damage. In subacute and chronic stages, tissue cysts are seen in mesenchymal host cells and are described in a variety of tissues, including being frequently described in the skin.[5]

In this case, the primary lesions, aside from the cysts, include interstitial nephritis, fibrosis, and both tubular and glomerular damage. The interstitial infiltrate is diverse, ranging from predominantly lymphocytes and plasma cells in some areas to being neutrophil- and eosinophil-rich in others. There is mild multifocal intratubular inflammation with tubular degeneration and necrosis. Glomerular changes include membranoproliferative glomerulonephritis and glomeruli range from essentially normal to obsolescent; periglomerular fibrosis is striking in some areas. Amyloid is present in small amounts multifocally within the medullary interstitium, and during the conference, was confirmed with Congo red staining and green birefringence under polarization. The moderator also discussed the presence of extramedullary myelo-



Kidney, opossum: Leptospires in the lumen of a proximal convoluted tubule. (Warthin-Starry 4.0, 1000X)

poiesis, which is multifocal and extensive in some areas of the cortical interstitium.

In light of the interstitial nephritis and tubular changes, conference participants considered leptospirosis as a primary differential diagnosis. A Warthin-Starry stain identified low numbers of leptospires in the lumen of renal tubules. The opossum has been documented as a reservoir for multiple leptospirosis serovars. Renal lesions due to leptospirosis vary with virulence of the infecting serovar and stage of infection, but generally consist of varying degrees of tubulointerstitial nephritis and tubular necrosis. Subacute or chronic cases have an increased degree of interstitial inflammation and fibrosis may be extensive.[4]

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

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CASE III: V15-04651 (JPC 4068934).

Signalment: Three-year-old female green iguana / common iguana (*Iguana iguana*)

History: The owner raises green iguanas as well as multiple other species of iguanas.

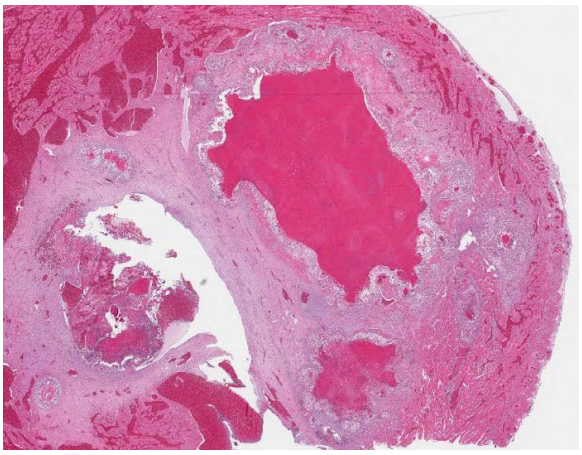
Heart, green iguana. Multiple cross-sections of the heart demonstrate a fibrinopurulent exudate filling the right ventricle and extending into the myocardium. Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services, 1101 Camino de Salud NE Albuquerque, NM 87102 www.nmda.nmsu.edu)

The three green iguanas consisted of two females and a male that were housed together. The green iguanas would fight amongst themselves with two of the iguanas developing cutaneous abscesses. The female iguana presented for necropsy became acutely ill and was dead on arrival at the submitting veterinary clinic.

Gross Pathology: The iguana was in good body condition with mild postmortem decomposition. There was a 1.8 x 1.8 cm subcutaneous abscess on the chin. The liver was enlarged and slightly pale. The spleen was enlarged. The urinary bladder contained large amounts of urates. The lungs contained a few multifocal pinpoint to 0.3 cm white foci. The pericardial sac contained moderate amounts of slightly cloudy light yellow fluid that contained free floating clots of fibrin. The pericardial sac was focally adhered to the epicardium of the left ventricle. The endocardium associated with the pericardial adhesion was thick and white. The lumen of the right ventricle was almost completely filled with a large, friable, yellow, fibrino-purulent exudate that was attached to the endocardium and multifocally extended into the myocardium. The endocardium of the right ventricle was thickened and white.

Laboratory Results: *Neisseria iguanae* was cultured from the subcutaneous abscess and the exudate in the right ventricle of the heart.

Histopathologic Description: Heart: The lumen of the right ventricle contains a large thrombus consisting of fibrin, cellular debris, and rare macrophages and heterophils that is multifocally attached to the mural and valvular endocardium. The thrombus contains moderate numbers of small gram-negative cocci. The thrombus is surrounded by numerous epithelioid macrophages and multinucleated giant cells with lesser numbers of heterophils and lymphocytes. The endocardium of the right ventricle is markedly thickened by fibrous tissue that contains small numbers of granulomas characterized by a center of fibrin, necrotic debris and small cocci surrounded by epithelioid macrophages, multinucleated giant cells, heterophils, and lymphocytes. There are a few similar granulomas in the myocardium of the right ventricle. The epicardium is multifocally



Heart, green iguana. Transverse section of the heart with a large luminal thrombus in the right ventricle with associated endocardial and subendocardial fibrosis. (HE, 2X)

thickened by fibrous tissue, macrophages, heterophils, and lymphocytes.

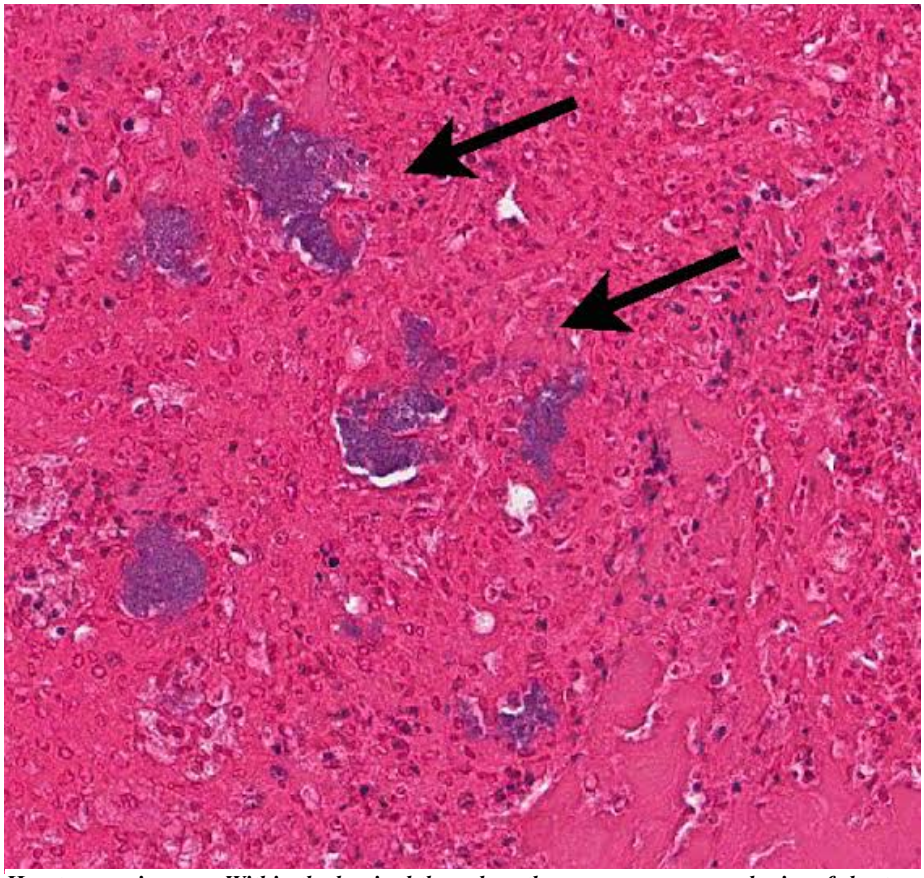
Contributor's Morphologic Diagnosis:

1. Heart, right ventricle: Mural and valvular vegetative endocarditis with heterophilic and granulomatous inflammation and intralesional small gram-negative cocci; etiology, *Neisseria iguana*

2. Heart, right ventricle: Myocardial granulomas

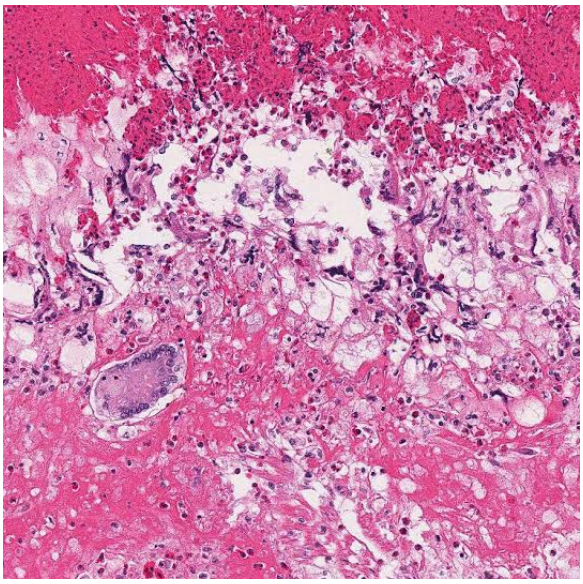
Contributor's Comment: *Neisseria* sp. are gram-negative bacteria that typically grow as diplococci in culture.[5,7] The most well-known species of *Neisseria* are *N. gonorrheae* and *N. meningitidis*. *Neisseria gonorrhea* is a venereal disease of humans. *Neisseria meningitidis* causes meningitis in people. However, most *Neisseria* species are commensal bacteria that are part of the normal oral and nasopharyngeal flora of mammals and the intestinal tract of birds.

In the 1980's, a syndrome of abscesses and septicemia in rhinoceros iguana and common iguana was identified at the National Zoological Park.[7] The causative agent of the disease was classified as a new bacterium called *Neisseria iguanae*.[1] The bacterium was also isolated from the oral cavity from healthy animals in the collection. The cutaneous abscesses were believed to be the result of *N. iguanae* infection of the skin following bite wounds. One of the iguanas developed *N. iguanae* septicemia manifested as a liver abscess. In this case, the intraspecies aggression between the three common iguanas most likely resulted in the cutaneous abscess and mural and valvular endocarditis caused by *Neisseria iguanae* in this case.



Heart, green iguana. Within the luminal thrombus, there are numerous colonies of short bacilli. (HE, 400X)

The pathogenesis of bacterial endocarditis (also classified as infective endocarditis) is complex.[4,8,9,10] The formation of infective endocarditis lesions involves the preparation of the endothelial layer for colonization, adherence of bacteria to the endothelial surface and survival of the bacteria with propaga-tion of the thrombus.[8] Intact endothelium is believed to be resistant to bacterial

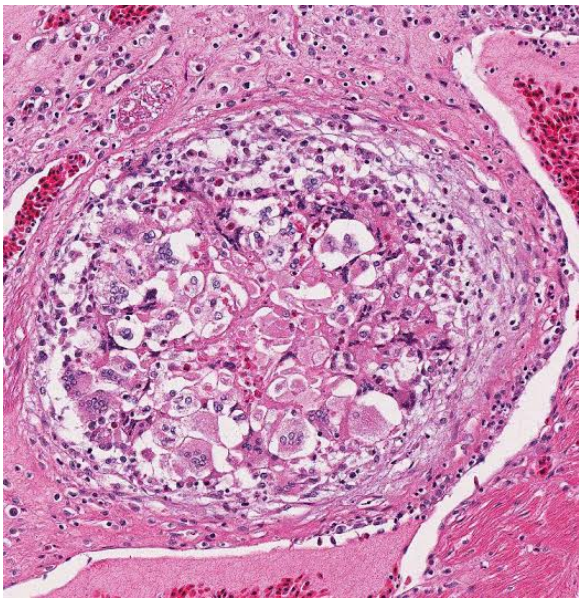


Heart, green iguana: The interface between the thrombus and adjacent endocardium contains numerous degenerating heterophils, epithelioid macrophages, foreign body type macrophages and abundant polymerized fibrin. (HE, 87X)

colonization.[4,8,9,10] The resistant endothelial layer has to be disturbed in order for bacteria to adhere. The disturbance of the endothelial layer can be the result of mechanical forces or due to endothelial cell activation and damage as the result of local proinflammatory molecules such as IL-1.[10] The endothelial cell damage causes activation of the coagulation cascade through the activity of tissue factor resulting in what is termed nonbacterial thrombotic endocarditis (NBTE). The resulting thrombus is colonized by bacteria that can adhere to damaged endothelial cells, platelets and adhesive extracellular matrix molecules such as fibrin and fibronectin. The bacteria adhere to the matrix molecules of the clot using a variety of surface molecules collectively called microbial surface component reacting with adhesive matrix molecules (MSCRAMMs).[10] The proliferation of the thrombus of infective endocarditis involves the interaction of bacterial pathogens and the host immune and coagulation systems. Infective endocarditis in the right side of the heart can result in emboli showering the lungs.[9] Infective endocarditis in the left side of the heart can result in systemic embolism.

JPC Diagnosis: Heart: Endomyocarditis, granulomatous and heterophilic, focally extensive, marked with ventricular thrombosis and numerous bacterial colonies.

Conference Comment: *Neisseria* spp. are generally classified as obligate human pathogens, commensal organisms in humans and mammals and/or organisms that may cause opportunistic human infections. As mentioned above by the contributor *Neisseria* spp. bacteria are common oral flora of many mammals including *N. canis*, which has been isolated from the throats of cats and can be present in cat bite wound infections, and *N. weaver*, *N. zoodegmatis*, *N. animaloris* which are normal oral flora of dogs and can be present in dog bite wound infections and in some cases can result in systemic infections in humans. Other species have been isolated from the oral cavity of guinea pigs, cows and rhesus monkeys. *Neisseria* spp. have also been isolated from the duodenum of healthy cats. Pathogenic *Neisseria* spp. utilize a number of adhesins, most commonly referenced with regard to human infections, and one of the best known is the type IV pilus which imparts twitching motility and facilitates uptake of foreign DNA. Most of the *Neisseria* spp. causing significant disease also possess a polysaccharide capsule, enabling avoidance of complement mediated killing and phagocytosis. Another feature which aids in resistance to antibody and complement mediated killing includes lipo-oligosaccharide (LOS), which is a membrane structure composed of lipid and oligosaccharide which is structurally different from lipopolysaccharide (LPS).[3]



Heart, green iguana. Within the fibrotic endocardium and adjacent myocardium, there are numerous well-formed granulomas. (HE, 58X).

Conference participants described this lesion as a severe granulomatous endocarditis with granuloma formation and fibrosis. The ventricle was described as being 100% occluded by a large, dense fibrin thrombus which contains numerous bacterial colonies as well as erythrocytes and necrotic debris, and is multifocally attached to the markedly thickened endocardium. Multiple granulomas are present in the superficial myocardium, near the epicardial–myocardial junction. Granulomas contain a dense core of eosinophilic debris (characteristic of reptile granulomas) surrounded by multiple macrophages with the presence of many multinucleate giant cells as well. The abundant white space surrounding the dense central core of debris is likely the result of retraction artifact. The granuloma's most peripheral layer is composed of dense fibrous connective tissue. The differential diagnosis discussed includes mycobacterial and fungal infections.

The moderator briefly discussed the structure of reptile hearts as there are significant differences with mammalian hearts. Most reptiles have a single common ventricle and two atria. Three cavities or divisions are present in the ventricle, termed the *cavum pulmonale*, *cavum arteriosum* and *cavum venosum* and are partially separated by muscular septa. Blood flows from the right atrium, through the cavum venosum and into the cavum pulmonale and then enters the pulmonary circulation. Oxygenated blood flows from the pulmonary veins and reenters the heart through left atrium, flows into the cavum arteriosum during diastole, which channels blood into the cavum venosum which then flows into the aorta. Oxygenated and de-oxygenated blood is separated by pressure differences, outflow resistance and differential flow. Shunting and mixing of oxygenated and deoxygenated blood is variable depending on the reptile species and activity level. Nonetheless, blood flows are described as well separated within the ventricle (due to septa) and mixing of oxygen-poor and oxygen-rich blood is minimized.[4]

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CASE IV: E6400/14 (JPC 4066312).

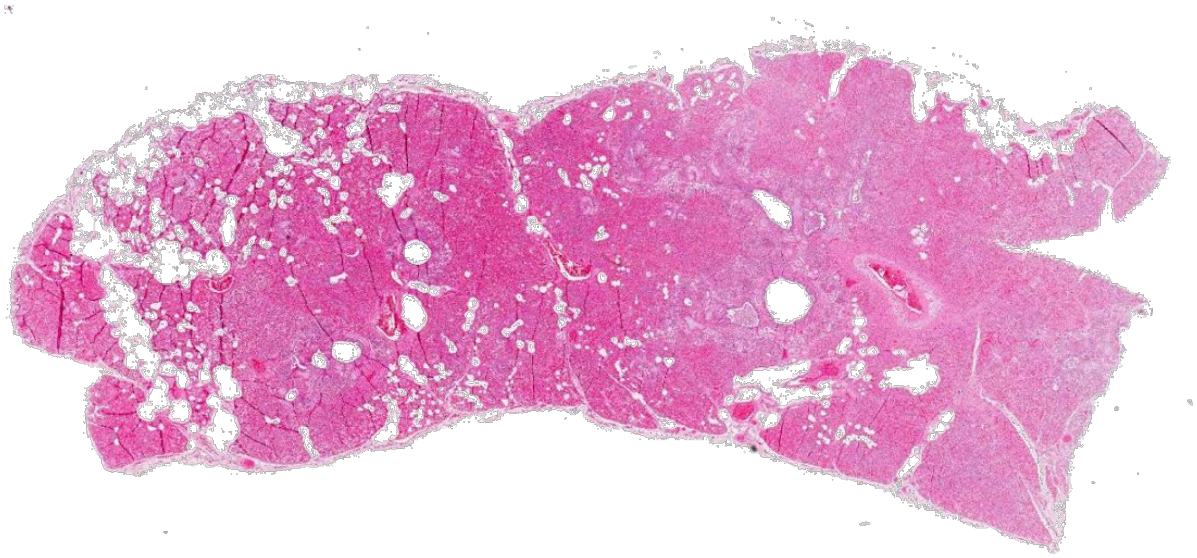
Signalment: Juvenile, male harbor seal (*Phoca vitulina*).

History: In 2014, an increased mortality occurred among harbor seals (*Phoca vitulina*) in northwestern European waters. Hundreds of carcasses were washed up on the shores of Sweden, Denmark, and Germany.[4, 10, 17] Along the Wadden sea coast of Lower Saxony, Germany, more than 320, and along the coast of Schleswig-Holstein, Germany, more than 2100 dead harbor seals were counted by the end of 2014.[11] The population size in the German part of the Wadden sea is approximately 12,000 animals. [19] This necropsied seal was found moribund on the beach of the north Freisian coast close to the town of Büsum. The animal was euthanized humanely.

Gross Pathology: Necropsy revealed a poor nutritional status and severe generalized muscular atrophy. The poorly retracted lungs displayed severe congestion, diffuse consolidation, and multifocal firm nodular areas of gray-yellow discoloration with varying numbers of metazoan parasites. Additionally, there was severe, diffuse alveolar and interstitial emphysema. The pulmonary lymph nodes were markedly enlarged, and the tonsils were moderately swollen. There was no content in the stomach.

Laboratory Results: Lung tissue and tracheal swab were positive for influenza A virus using an influenza A-specific PCR for the matrix protein. Virus culture on embryonated chicken eggs and Madin-Darby canine kidney (MDCK) cells resulted in isolation of influenza A virus. Using specific primer sets for the hemagglutinin and neuraminidase genes with subsequent sequencing of the amplicons allowed classification into the subtype H10N7 of the influenza A virus.

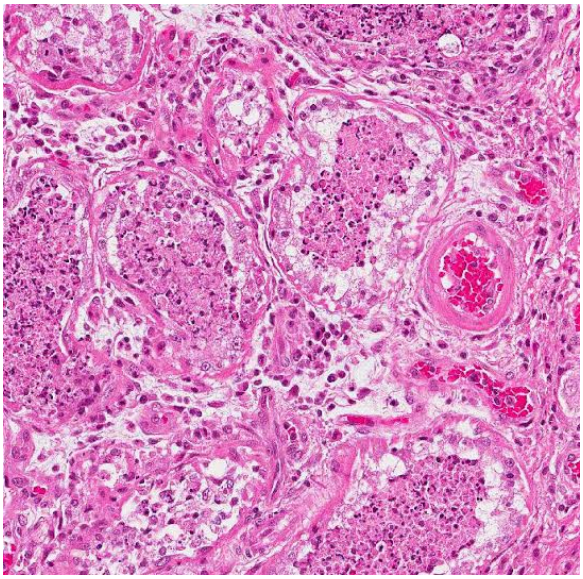
Immunolabeling of influenza A virus nucleoprotein revealed specific antigen staining in the respiratory tract.



Lung, harbor seal. At subgross inspection, there is diffuse consolidation and moderate segmental emphysema along the interlobular septa and pleura (HE, 4X).

Immunohistochemistry for morbillivirus nucleoprotein and reverse transcription PCR for morbillivirus were negative.

Microbiologically, moderate colony numbers of *Escherichia coli* and low numbers of *Acinetobacter pittii, Streptococcus phocae and Stenotrophomonas maltophila* were isolated from the lung.



Lung, harbor seal. There is extensive necrosis within submucosal glands around large airways. (HE, 120X)

Parasitologically, the lung worms *Otostrongylus circumlitus* and *Parafilaroides gymnurus*, as well as the heartworm *Acanthocheilonema* (previously termed *Dipetalonema*) *spirocauda*, were identified in the lungs.

Histopathologic Description: The tissue from the lung displays a mild diffuse hyperplasia of bronchial epithelial cells with loss of cilia. There are mildly scattered karyopyknotic cells (necrosis) within the epithelial lining of the deep airways associated with few intraepithelial lymphocytes and occasional mitotic figures. Bronchial lumina are partially filled with cellular debris, neutrophils, foamy macrophages and desquamated epithelial cells. Bronchial glands show severe hydropic swelling, karyorrhexis and pyknosis of epithelial cells (degeneration and necrosis) with accumulation of cellular debris within the lumina. In the peribronchial and perivascular interstitium, there is a moderate to severe infiltration of lymphocytes, plasma cells and macrophages extending multifocally into the interalveolar interstitium. Most alveolar lumina are filled either with macrophages and desquamated

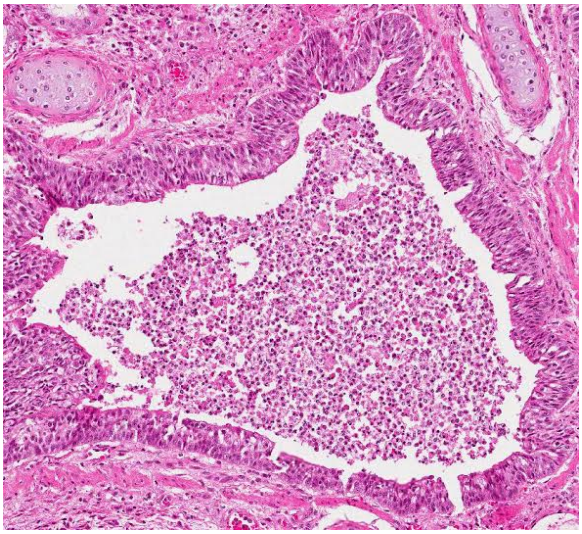
pneumocytes or neutrophils and macrophages. Occasionally, few to moderate numbers of erythrocytes are present in alveolar lumina. Furthermore, accumulation of eosinophilic homogenous or fine fibrillar material is present in alveoli (fibrin), sometimes in a membranous shape, along the alveolar wall. Interalveolar capillaries are engorged with red blood cells. Multifocally, venous vessels show partial occlusion by eosinophilic material attached to the vascular wall with infiltrating fibroblasts and endothelial lining on the surface. Multifocally, there are cross and longitudinal sections of larval nematode structures with a diameter of up to 50 µm and a length of about 200 µm surrounded by a cuticle. In the body cavity, a digestive tract is present. Restricted to few lobules, there are distended alveoli with ruptured interalveolar septae as well as distended interlobular septae.

Contributor's Morphologic Diagnosis:

Lung: Pneumonia, broncho-interstitial, moderate, chronic, multifocal, lympho-histiocytic and plasmacytic with hyperplasia of bronchial epithelium; bronchitis, moderate, acute, multifocal, necrotizing with necrotizing adenitis of bronchial glands; pneumonia, suppurative, multifocal, moderate; multifocal subtotal vascular thrombosis; pulmonary endoparasitosis with larval stages of nematodes.

Contributor's Comment: The seal suffered from severe pneumonia caused by a concurrent infection with influenza A virus H10N7, pulmonary endoparasites, and several bacterial species. Besides the lung, morphologic changes associated with the influenza A virus infection were present only in the upper respiratory tract characterized by acute necrotizing rhinitis and tracheitis. Immunohistologically, influenza A virus nucleoprotein was demonstrated intralesionally in the cytoplasm and nuclei of epithelial cells of bronchi and bronchial glands. Additionally, it was present in nasal and tracheal respiratory epithelium.

Avian influenza A viruses are known to cross species barriers and infect various mammalian species, including man and pinnipeds.[2, 4, 9,10,13] Different influenza virus

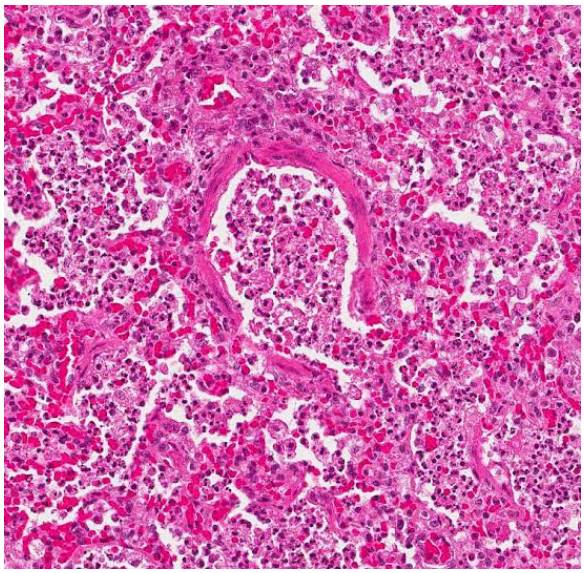


Lung, harbor seal. Large airways are filled with a cellular exudate composed of sloughed epithelium, histiocytes and neutrophils admixed with cellular debris (likely representing reflux from surrounding alveoli.). There is mild hyperplasia of airway epithelium, which is infiltrated by low numbers of neutrophils. (HE, 87X).

A subtypes have previously been reported to cause fatal mass mortalities of harbor seals, including subtypes H5N1, H3N8 and H5N4.[2, 4, 9,13] However, subtype H10N7 has not been isolated from seals before. Infections with the subtype H10N7 have been reported in man in Egypt and Australia,[3, 7] and various avian species including chicken,[3,14] ducks,[14] mallards,[17] and wild birds.[16] According to the predicted amino acid sequence, this subtype is regarded as a low pathogenic avian influenza (LPAI) strain with a low zoonotic potential.[11, 17] However, virus detection has been reported in spleen samples of affected seals from Denmark and a more virulent

potential of this H10N7 subtype cannot be excluded completely.[11]

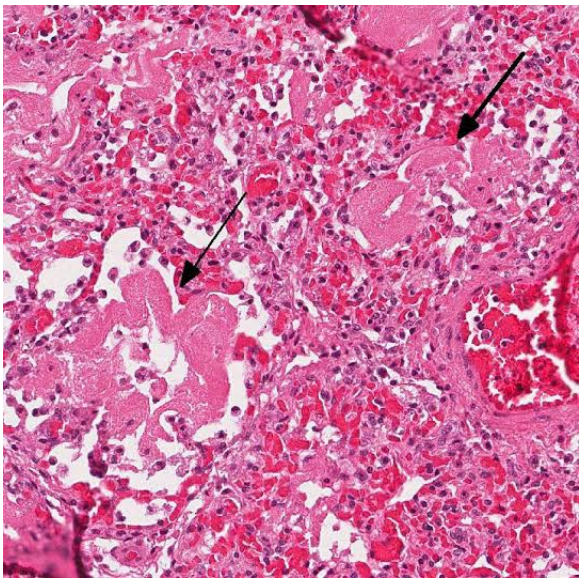
The influenza epidemic in 2014 in northern European waters started with an increased mortality among harbor seals in March in Sweden, and swapped in July to Denmark,[11] and reached Germany in October and The Netherlands in November. More than 2400 dead harbor seals were counted in German coastal waters.[4, 12] The origin and mode of transmission of this avian influenza virus remains undetermined. Phylogenetic analysis revealed a close relationship to various influenza A viruses detected in wild birds. Specifically, the hemagglutinin and neuraminidase genes were genetically most



Lung, harbor seal. Alveolar septa are diffusely expanded by congestion, small amounts of edema and fibrin, circulating neutrophils, and patchy type 2 pneumocyte hyperplasia. (HE, 120X)

closely related to subtype H10N7 viruses recently found in migratory ducks in Georgia, Egypt, and the Netherlands.[4] The seals may be infected oro-nasally with the virus through direct or indirect contact with wild birds or their droppings, because they share the same shoreline habitats as waterfowl.[11, 18] Hemagglutinin is the attachment protein of influenza viruses, binding to sialylated glycans on the host cell surface. Avian and swine influenza viruses bind to α2,3 linked sialic acid. In contrast, human and several mammalian influenza A viruses bind to sialylated glycans with an α2,6 linkage to galactose.[1,2]

Virus-associated destruction of epithelial cells in the respiratory tract probably caused a predisposition for secondary bacterial infection resulting in suppurative pneumonia. Together with the parasitic burden in the lung, respiratory insufficiency is regarded as a main cause of death. Bacteriologically, *Escherichia coli, Acinetobacter pittii, Streptococcus phocae* and *Stenotrophomonas maltophila* were isolated in the present case. *Acinetobacter pittii* and *Streptococcus phocae* are regarded as opportunistic pathogens. Similarly, *Streptococcus phocae* has been isolated from seals affected with phocine distemper, but has also been associated with starvation and abortion in Cape fur seals (*Arcto-cephalus pusillus pusillus*).[7] Interestingly, bacteriological culture of lung samples from other seal carcasses with influenza A H10N7 infection resulted in isolation of bacteria, e.g. *Bordetella bronchiseptica* and *Streptococcus equi* ssp. *zooepidemicus* that were not isolated from lung samples of seal carcasses investigated during the annual health monitoring program. However, these bacterial species were exclusively isolated during the seal die-offs in 2002 and 2014 as a secondary pathogen (Siebert, pers. communication).



Lung, harbor seal. Multifocally, damaged alveolar septa have flooded the lumina with fibrin which has organized into thick hyaline membranes (arrows). (HE, 160X)

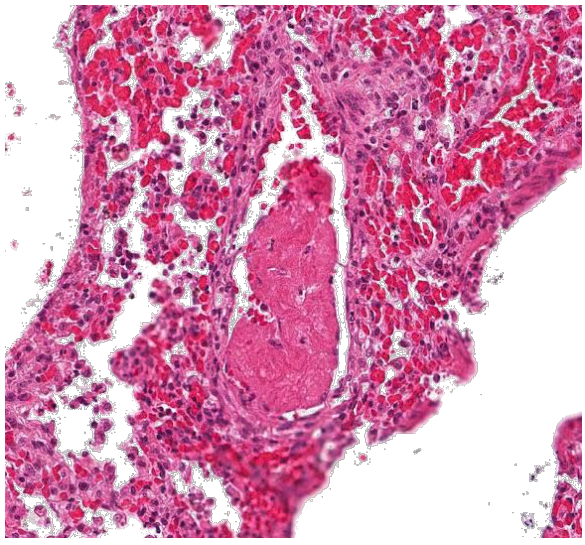
Commonly reported nematodes in the lung of harbor seals are *Parafilaroides gymnurus*, *Otostrongylus circumlitus*, and the heartworm *Acanthocheilonema* (previously termed *Dipetalonema*) *spirocauda*.[15] Nematodic infections of the respiratory tract of harbor seals represent a common finding, particularly in juvenile individuals.[15] The epithelial hyperplasia of deep airways, as well as the vascular thrombosis, may be related to the parasitic infestation in this case.

As differential diagnosis for infectious seal mass die-offs, phocine distemper virus (PDV) infection has to be considered. Phocine distemper was excluded in this case by reverse transcription PCR (RT-PCR) and immunohistochemistry. The total Wadden Sea seal population has reached a so far unprecedented population size of more than 39,000 animals. A serological survey revealed a lack of antibodies against phocine distemper indicating a high susceptibility for phocine distemper.[6] The current prevalence of influenza virus antibodies in the German harbor seal population is unknown.

JPC Diagnosis: 1. Lung: Pneumonia, bronchointerstitial, necrotizing and fibrinous, diffuse, severe, with submucosal gland necrosis, alveolar and interlobular emphysema, and organizing fibrin thrombi.

2. Lung: Larval nematodes, few (variable across sections).

Conference Comment: Influenza A virus affects many species and waterfowl are considered the natural reservoir host for many subtypes. Subtypes which result in infection of mammals often occur through transmission from waterfowl. These viruses replicate in the intestinal tract of birds and fecal-oral transmission is the primary route of infection. In a rash of harbor seal deaths in Denmark reported in conjunction with the outbreak from which this case originated, (discussed above) animals were also affected with a necrotizing bronchopneumonia with the presence of bacteria in alveoli. Influenza virus A (H10N7) was also isolated in those cases; additionally, bacterial isolates included *Pseudomonas aeruginosa* with variable growth of *Streptoccus equi* subsp. *zooepidemicus*. The pulmonary lesions were attributed to

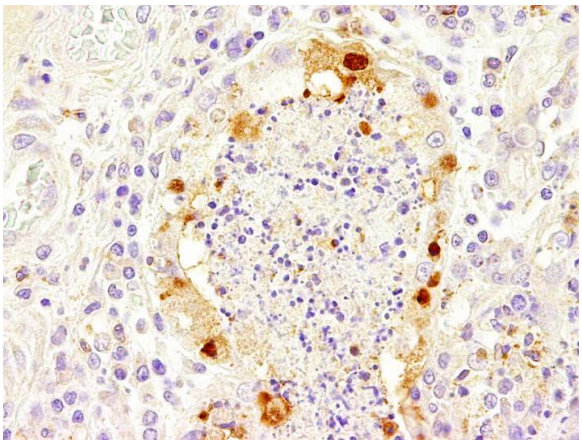


Lung, harbor seal. Occasionally vessels contain fibrin thrombi. (HE, 120X)

primary infection with influenza A, complicated by secondary bacterial infection, as seen in this case.[11]

During the outbreak described above, which primarily resulted in seal deaths in Sweden, Denmark and Germany, only low numbers of seals were found dead in the Netherlands; the precise reason for variable mortality is unclear. A study measuring antibody levels of H10N7 (which in some references is termed seal influenza A) in captured or rehabilitated seals in the Netherlands found detectable antibodies in 41% of pups, 10% of weaners and 58% of adults or subadults, indicating infection with this virus may be widespread. Antibody titers were also found in adult grey seals. In subadult harbor and grey seals sampled prior to the 2014-2015 outbreak, only a small number had antibody titers against the virus, which may indicate absence of widespread herd immunity at the start of the outbreak.[5]

The conference histologic description was similar to the contributor's histologic description above. There was extensive discussion regarding the vascular changes in the section. There are distinctive fibrin thrombi partially occluding vessel lumina, but there are also vessels which appear to have near complete occlusion by fibroblasts and/or macrophages that are surrounded by concentric layers of fibrous tissue. Participants postulated the vascular changes may represent variable chronicity of the lesions, with the older, mature thrombi demonstrating partial to complete recanalization. Multifocally, hyaline membranes line the alveolar surface and are more prominent in the less atelectatic areas of the lung. Alveolar and interlobular septal emphysema were also described. Multifocally there is hyperplasia of bronchial and bronchiolar epithelium, with loss of cilia in many areas. The contributor provides a detailed description and excellent history regarding the events surrounding this case.



Lung, harbor seal. Labeling of influenza A nucleoprotein in submucosal gland epithelium. (anti-influenza A with HE counterstain, 400X)

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