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United States Army Medical Research Institute
of Infectious Diseases (USAMRIID)
Fort Detrick, MD

CASE I – 98-4356 (AFIP 2681370)

Signalment: Adult, male, timber rattlesnake (*Crotalus horridus*).

History: Five of 7 rattlesnakes within an exhibit have died and the remaining 2 are sick. The snakes are bloated, off feed and have seizures.

Gross Pathology: Little body fat is present. The coelom contains caseous material. There are numerous 1 mm white foci in the coelom and the lung.

Contributor's Morphologic Diagnoses: 1. Liver: Cytoplasmic inclusions, hepatocytes.
2. Kidney: Nephritis, interstitial, lymphocytic, multifocal, mild with cytoplasmic inclusions in tubular epithelial cells.
3. Lung: Pneumonia, necrotizing, purulent and granulomatous, with rare intraepithelial cytoplasmic inclusions and numerous bacteria.

Contributor's Comment: This is a case of paramyxovirus infection in rattlesnakes. A secondary bacterial pneumonia is the predominant lesion in this case and a bacterial coelomitis was also present. Ophidian paramyxovirus infection has been recently described in snakes and produces immunosuppression like the mammalian virus. Secondary bacterial infections are common and are the usual cause of death. The lung and nervous system are the usual organs affected but secondary bacterial infections can occur in a variety of organs. Intracytoplasmic viral inclusions are seen in a variety of epithelial cells but formation of syncytial cells, as occurs in mammalian paramyxovirus infections, is rare.

AFIP Diagnoses: 1. Lung: Pneumonia, bronchointerstitial, granulomatous and heterophilic, diffuse, severe, with multifocal necrosis, epithelial hyperplasia, and rare epithelial eosinophilic intracytoplasmic inclusion bodies, etiology consistent with ophidian paramyxovirus, timber rattlesnake (*Crotalus horridus*), reptile.
2. Kidney: Nephritis, interstitial, lymphocytic and histiocytic, subacute, multifocal, mild, with tubular epithelial degeneration and necrosis.
3. Esophagus, epithelium: Necrosis, multifocal, with rare eosinophilic intracytoplasmic inclusion bodies.

Conference Comment: The respiratory system of snakes is similar to that of mammals with a few distinct differences. The left lung is vestigial in all snakes except boas. The right lung has a posterior avascular portion, known as the air sac. The air sac regulates pressure inside the body cavity. The anterior portion of the lung is composed of faveoli, similar to mammalian alveoli, separated by relatively thin septae that are lined by capillaries and type I and type II pneumocytes. Since snakes do not have a diaphragm, air enters and leaves the lung due to action of the body muscles and movement of the ribs.[3]

Ophidian paramyxovirus (OPMV) is a single-stranded enveloped RNA virus and is an extremely important pathogen of viperid snakes. There have also been reports of OPMV in colubrid, boid, and elapid snakes. Clinical signs typically include loss of muscle tone, abnormal behavior and dilated pupils. However, in many of the outbreaks of OPMV, the snakes are found dead with minimal or no clinical signs noted.[4]

The most significant gross lesion of OPMV disease is hemorrhage of the lung and air sac with caseous necrotic debris. Additional gross lesions include pancreatic hyperplasia, and hepatic granulomas or caseous necrosis. Typical microscopic findings include cellular debris and exudate within airways, type II pneumocyte hyperplasia, thickening of faveolar septa, and few epithelial cells containing intracytoplasmic inclusion bodies.[4]

Like mammalian paramyxoviruses, OPMV also causes immunosuppression. Animals infected with OPMV, as in this case, commonly have secondary bacterial infections with gram-negative organisms. The most common gram-negative organisms isolated from reptiles with pneumonia include *Pseudomonas* spp., *Providencia* spp., *Proteus* spp., *Salmonella* spp., *Aeromonas hydrophila*, and *Escherichia coli*. Thus, it is not surprising that snakes infected with OPMV often succumb to secondary bacterial pathogens.[4]

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CASE II – PP1481D (AFIP 2936457)

Signalment: 1.5 year old, female, harbor porpoise (*Phocoena phocoena*), cetacean.

History: A juvenile, female harbor porpoise, weighing 41 kg, was rescued in April 1999 from a pound net close to Baaring Vig and taken to the Fjord and Belt Centre, Kerteminde for rehabilitation. The animal was regularly treated with anthelmintics and clinically examined. A couple of weeks later, the porpoise developed pox-like lesions on the skin. The lesions persisted for 6 to 8 months but healed without scars. On 12th February 2000, the porpoise developed clinical signs of illness consisting of reduced appetite, floating on the surface and being unable to support itself in the water. The animal had two crises, with high respiratory rate and low body temperature. The porpoise was treated intensively, including antibiotics. In spite of intensive medical care, the animal died on February 22nd, 2000 after 10 days of illness.

Gross Pathology: The porpoise was in a good nutritional status. There was an acute fibrinous to suppurative pleuritis and pericarditis with approximately 0.4 to 1 liter of exudate. One myocardial abscess, approximately 5 cm in diameter, was found in the left ventricle with fistulous tracts to both, the endocardium and epicardium. Another myocardial abscess, 0.5 cm in diameter, was located at the base of the heart. There was a severe diffuse, fibrinous, suppurative peritonitis with 1 litre of exudate. In the esophagus few ulcerations were detected. On the skin of the left side of the body there were multiple round foci approximately 1 cm in diameter consisting of a white center surrounded by a black rim. In the bronchial tree and pulmonary blood vessels a mild to moderate parasitic infection with *Pseudalius inflexus* and *Torynurus convolutus* as well as a moderate parasitic burden of *Stenurus minor* in both aural peribullar cavities were observed. In the

first compartment of the stomach one nematode (*Anisakis simplex*) and a few fish eyes were found. In the second compartment of the stomach few submucosal hemorrhages were noted with a diameter of about 0.5 cm. In the right lower jaw, the third tooth was dislocated towards the buccal gingiva.

Laboratory Results: *Staphylococcus aureus* was cultured from a sample taken from the blowhole while the animal was alive. Postmortem samples from cardiac abscesses, liver, spleen, and kidneys yielded *Staphylococcus aureus*. In addition, a few colonies of streptococci and *Serratia liquefaciens* were found. Skin biopsies revealed moderate cytoplasmic swelling and vacuolization of keratinocytes; however, ultrastructurally pox virus particles could not be detected. Morbillivirus antigen was not detected by immunohistochemistry.

Contributor's Morphologic Diagnosis: 1. Heart: Myocarditis, pyogranulomatous, chronic, multifocal to coalescing, severe with necrosis, colonies of cocci, homogenous eosinophilic material (consistent with Splendore-Hoeppli material), fibrosis, harbor porpoise (*Phocoena phocoena*), cetacean.
2. Heart: Myocarditis, lymphocytic, chronic, multifocal, mild.
3. Heart: Epicarditis, lymphohistiocytic, chronic, diffuse, moderate.

Contributor's Comment: The submitted tissue section includes myocardium, epicardium and fibrous connective tissue of a harbor porpoise. The main lesion consists of a multifocal extensive myocardial necrosis with myriad cluster-forming cocci which are surrounded by homogenous eosinophilic substance in a club-shaped radiating corona (consistent with Splendore-Hoeppli material). The surrounding tissue is markedly infiltrated with inflammatory cells, predominantly neutrophils, macrophages and epithelioid macrophages admixed with lymphocytes and plasma cells. The periphery of the lesion is demarcated by a poorly vascularized fibrous tissue. Multifocal areas of the myocardium showed a mild, interfascicular infiltration of lymphocytes. The epicardium was irregularly thickened by an infiltration of lymphocytes and histiocytes.

Upon histological examination of other tissues, a multifocal acute hepatocellular, centrolobular necrosis without inflammatory reaction, and chronic suppurative pericholangitis, were detected. Follicular hyperplasia was found in the spleen. In the adrenal gland a mild focal lymphoplasmacytic infiltration with multifocal hemorrhages was observed.

Staphylococcus aureus was isolated from the cardiac abscess and other organs. Chronic infections caused by *Staphylococcus aureus* are termed botryomycosis. This is a poorly understood pyogranulomatous bacterial disease of the skin and subcutis or, more rarely, the viscera. In humans, cutaneous, pulmonary, and hepatic botryomycosis have been reported. In veterinary medicine botryomycosis

is recognized as a staphylococcal wound infection in horses and pigs. In addition, pulmonary botryomycosis has been reported in horses and guinea pigs.[3] In cattle, swine and elephants it is an unusual manifestation of mastitis.[1,2] One case of disseminated staphylococcal botryomycosis has been described in a cat with perforating gastric ulcer.[4] Intraabdominal botryomycosis has been diagnosed in a dog.[5] In two harbor porpoises (*Phocoena phocoena*), one case submitted here, a pyogranulomatous myocarditis due to *Staphylococcus aureus* septicemia has been reported.[6] In three harp seals (*Pagophilus groenlandicus*) the presence of subcutaneous and systemic botryomycosis was described.[7]

The pathogenesis of botryomycosis is still unclear. An imbalance between virulence of the organism and host resistance may result in incomplete removal of bacteria by the host which leads to formation of bacterial granulomas.[8] The exact composition of the infiltrating population of inflammatory cells depends of the stage of the infection. At initial examination, the lesion may have a purulent exudate with small white granules with a diameter of less than 1 mm. These granules are indistinguishable from those of actinomycosis, nocardiosis, and mycetoma unless special stains are used. In botryomycosis the capsules of the grains were homogenously eosinophilic (Splendore-Hoeppli material), periodic acid-Schiff (PAS) positive, slightly acid-fast, weakly positive with Gomori's methenamine silver stain, and were unstained with the MacCallum-Goodpasture modification of the Gram stain.[3]

Differential diagnoses include systemic mycosis, chronic bacterial abscesses, and foreign body reaction. Fungal hyphae are best demonstrated with silver stains (for example Grocott's stain). *Actinomyces* and *Nocardia* are Gram-positive filamentous organisms, and acid-fast in the case of *Nocardia* (for example Ziehl-Neelsen stain). Most reported cases of botryomycosis are caused by *Staphylococcus aureus*, but *E. coli*, *Pseudomonas aeruginosa*, *Actinobacillus lignieresii*, *Bacteroides*, *Streptococcus* and *Proteus species* have also been implicated.

Staphylococcus aureus is an uncommon finding in marine mammals. Some authors described *Staphylococcus aureus* as a commensal in the blowhole flora, whereas others classify it as a potential pathogen in clinically normal dolphins.[9] In the literature *Staphylococcus aureus* was described in association with septicemia in a killer whale,[10] a cerebral abscess in a dolphin,[11] and a septic embolic nephritis in the same species.[12] Furthermore it was described as a pathogen in pneumonia in dolphins,[13] cutaneous lesions, subcutaneous abscesses, and omphalitis in pinnipeds. In this case, the origin of the staphylococci remained unclear; the potential port of entry might be the pox-like lesions.

AFIP Diagnosis: 1. Heart: Myocarditis, pyogranulomatous, multifocal to coalescing, severe, with fibrosis, Splendore-Hoeppli material, and numerous colonies of cocci, harbor porpoise (*Phocoena phocoena*), cetacean.
2. Heart: Epicarditis, lymphoplasmacytic and histiocytic, chronic, diffuse, moderate.

Conference Comment: The contributor provides a thorough overview of staphylococcal infections and botryomycosis. As mentioned above, botryomycosis has been reported in many species and in a variety of organ systems, including the skin (humans, horses, pigs), lungs (horses, guinea pigs), mammary gland (cattle, pigs, elephants), stomach (cat), abdomen (dog), and most recently the heart (harbor porpoise).

Another large group of animals commonly affected by staphylococcal botryomycosis is laboratory animals, including mice, rats, gerbils, guinea pigs, hamsters, and rabbits. Staphylococci are common inhabitants of the skin and mucous membranes and are often carried asymptomatically. Many factors, such as immune status, lack of competing bacteria, nutritional deficiencies, trauma to the skin, and prevalence of staphylococci in the environment are recognized as contributing factors.[14]

Clinical signs in mice include abscessation of the cervical lymph nodes, inflammation and abscessation of the preputial and lacrimal glands, conjunctivitis, periorbital abscesses, superficial pyoderma, and severe ulcerative dermatitis. B6 mice are prone to ulcerative dermatitis, while nude mice tend to develop furunculosis around their muzzles, lacrimal gland abscesses, and preputial gland infections. In rats, staphylococcal ulcerative dermatitis characteristically is localized to the dorsal neck and interscapular regions. In young gerbils, *S. aureus* causes a diffuse moist dermatitis involving the face, nose, feet, legs, and ventral body surface. The nasal dermatitis in gerbils is associated with porphyrin-containing lacrimal gland secretions. When these secretions accumulate on the external nares, they act as a chemical irritant which leads to scratching, hair loss, and dermatitis. In guinea pigs, staphylococcal infections lead to ulcerative pododermatitis, also known as bumblefoot. Predisposing factors include trauma due to defective caging and poor sanitation. However, guinea pigs may also develop acute staphylococcal dermatitis (exfoliative dermatitis), which most frequently involves strain 13 guinea pigs. This disease is characterized by alopecia and erythema on the ventral abdomen with exfoliation of the epidermis. In hamsters, cutaneous and cervical abscesses are colonized by a variety of organisms including *S. aureus*, *Actinomyces bovis*, *Streptococcus* spp., and *Pasteurella pneumotropica*. Outbreaks of staphylococcosis occur sporadically in commercial rabbitries, with disease varying from localized abscessation to acute, and frequently fatal, septemia. In rabbits, lesions may occur in the skin, mammary

glands, genital tract, conjunctiva, footpads, and respiratory tract. The acute septemic form typically occurs in suckling kits during the first week of life and leads to multifocal suppurative lesions in the subcutaneous tissue, lung, kidney, spleen, heart, and liver.[14]

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CASE III – 05-0119 (AFIP 2964502)

Signalment: 1 + year old, male, Siamese fighting fish (*Betta splendens*).

History: Prior to euthanasia, Calvin had a two-month history of behavioral changes, hiding in his rock cave, and a three-week history of progressive coelomic enlargement.

Gross Pathology: Coelomic organs are displaced ventrally and caudally by a 1.2 cm diameter mass that occupies two-thirds of the centrodorsal portion of the coelom. The mass is composed of a cystic center filled with a viscous slightly opaque fluid and is surrounded by a 1-3 mm thick wall.

Contributor's Morphologic Diagnosis: Kidney: Nephroblastoma, Siamese fighting fish (*Betta splendens*).

Contributor's Comment: Nephroblastoma (Wilms' tumor, embryonal nephroma) occurs commonly in young pigs and chickens, less frequently in cattle and dogs, and is observed in various species of fish, amphibians and reptiles. It is the most common primary renal tumor in children (Marilyn J. Wolfe, personal communication).[1-3] Since these neoplasms arise from metanephric blastema, primitive pluripotential tissue, the triphasic histologic features resemble the developmental stages of embryonic kidneys with predominantly a myxomatous mesenchymal component interspersed with primitive tubules, embryonic glomeruli, occasional nests of cells resembling metanephric blastema, and rarely contain muscle, cartilage, bone and fat.[2,3]

The WT1 gene protein is a transcriptional activator of genes responsible for regulating cell growth and is essential in renal and gonadal differentiation and for the development of blastema into epithelium. Tissues that express WT1 are the uterus, spinal cord, spleen, abdominal wall musculature, mesothelium lining thoracic organs and the central nervous system. Mutations in the WT1 gene, a tumor suppressor gene, are associated with the development of Wilms' tumor in animals and humans. In addition, mutations in Beta-catenin, important in the *wnt* (*wingless*) signaling pathway, are demonstrated in 15% of humans with Wilms' tumor.[1,4] Thoracolumbar spinal cord tumors of young dogs that share similar histologic features and stain for the Wilms' tumor gene product are considered extrarenal nephroblastomas.[3]

This case was reviewed in consultation with Marilyn J. Wolfe, DVM, PhD, DACVP, Principal Investigator, Registry of Tumors in Lower Animals, Sterling, Virginia 20166-4311.

AFIP Diagnosis: Kidney: Nephroblastoma, Siamese fighting fish (*Betta splendens*), piscine.

Conference Comment: There is significant variation among slides and not all slides have all of the characteristic features of a nephroblastoma. As mentioned by the contributor, the diagnostic features of a nephroblastoma include the triphasic histologic features: myxomatous mesenchyme; interspersed primitive tubulesand/or glomerular-like buds; and, nests of cells resembling metanephric blastemain various amounts. Rarely, these tumors contain non-epithelial tissue such as muscle, cartilage, bone and fat.[3] In this case, the mesenchyme, primitive tubules, and blastema are present on all slides. However, there are very few glomerular-like buds and they are not present on all slides.

Adenocarcinoma of the swim bladder was considered by most residents. However, unlike many other fish, the swim bladder in Siamese fighting fish is located ventral to the kidney. Therefore, if the neoplasm originated from the swim bladder, one would expect that the unaffected kidney would be dorsal to the swim bladder. In all slides examined by conference participants, the unaffected portions of the kidney are ventral to the tumor. In many slides, it was difficult to determine if the tumor was originating within the kidney; however, in a few slides, it is clearly arising from the kidney.

In humans, nephroblastomas (Wilm's tumor) often present as a large, solitary, well-circumscribed mass; however, in 10% of the cases, the tumor is either bilateral or multicentric at the time of diagnosis. On cut section, the tumor is soft, homogeneous, tan to gray, with occasional hemorrhage, cyst formation and necrosis. Microscopically, the classic triphasic combination of blastemal, stromal, and epithelial cell types is observed in most tumors, although the percentage of each component is variable. Stromal elements are usually fibrocytic or myxoid, and skeletal muscle differentiation is not uncommon. Rarely, other elements, such as squamous or mucinous epithelium, smooth muscle, adipose tissue, cartilage and osteoid and neurogenic tissue are identified.[1]

This case was reviewed in consultation with Dr. Isabell A. Sesterhenn, Chair, Department of Genitourinary Pathology, The Armed Forces Institute of Pathology.

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CASE IV – G 6573/8 (AFIP 2956261)

Signalment: 1-year-old, female, Goeldi´s monkey (*Callimico goeldii*), non-human primate

History: For a period of several months the animal showed oral, perioral and nasal alterations which were characterized by swelling, edema, emphysema and incrustation. Treatment consisted of antibiotic therapy and regular removal of the overlying crusts to ease breathing, but clinical recovery could not be achieved. The swollen and hyperemic lips were still remarkable when the animal was necropsied after its accidental death. Retrospectively, similar clinical signs were present in both parents.

Gross Pathology: At necropsy the young Goeldi´s monkey was in poor nutritional condition and showed marked abdominal edema. Main post mortem findings were located in the upper respiratory and gastrointestinal tracts. Both nasal and oral tissues were swollen and edematous and the nose was covered with crusts. The overgrowing crusts and hyperemic lesions were mainly present on the mucocutaneous membranes of the lips and the nasal region. Numerous whitish nematodes up to 1 cm in length could be detected macroscopically within the mucous membranes of the lips, tongue, pharynx and intestine.

Laboratory Results: Bacteriological examination of a swab that was taken from the altered tissue - *Staphylococcus* sp. was cultured. Routine flotation of a fecal sample was negative for parasites and parasite eggs.

Contributor's Morphologic Diagnosis: 1. Skin (mucocutaneous junction): Dermatitis, minimal to mild, chronic, diffuse, with irregular epidermal hyperplasia and parasitic structures consistent with *Spirurida*, Goeldi´s monkey, non-human primate.
2. Intestine (not present in all slides): Several parasitic structures consistent with *Spirurida* within the lumen of the duodenum, Goeldi´s monkey, non-human primate.

Contributor's Comment: Histologically, the dermatitis is characterized by an irregular epidermal hyperplasia forming prominent rete ridges and pseudocarcinomatous proliferations in several locations. Focal erosions are present on the skin surface. In consequence of the parasite infection there is a mild lymphocytic inflammatory reaction associated with spongiosis in the superficial parts of the dermis. The parasitic structures are located within the epidermal layer where they are arranged in cystic cavities. In sections the nematodes do not cause any inflammatory response in the adjacent tissue. No parasites are seen inside the hair follicles.

The parasites were identified as nematodes of the order *Spirurida.* Characteristic histologic features are the muscular and glandular esophagus, lateral cephalic alae, the morphology of the lateral chords and the presence of embryonated eggs.[1] In the present case, further classification was not possible on the basis of histological examination. Because of the gross pathologic findings and the site of infection it is presumed that the parasites belong to the genus *Gongylonema*.

Spirurids of the genus *Gongylonema* are parasites of the upper digestive and respiratory tract in a variety of birds and mammals including non-human primates. Coprophagous arthropods (dung beetles and cockroaches) serve as intermediate hosts in the indirect life cycle. Embryonated eggs are deposited by the female worms, and liberated after epithelial desquamation with the host's feces. The first-stage larvae hatch in the insect's intestines, migrate into the body cavity and develop into the second larval stage after encapsulation. The maturation of the infectious third-stage larvae is completed after about four weeks. Final hosts acquire the parasite infection by ingestion of infected intermediate hosts or by drinking contaminated water. The migration pathway in the definitive host is still unknown for the most part, especially in primates. The larvae are released from their capsules under the influence of gastric acid and probably migrate within the wall of the upper intestinal tract, where they develop into adult worms.[2,3]

Gongylonema are considered to be non-pathogenic in most host species. Generally there is little tissue reaction with no extensive lesions. By contrast, reports about gongylonemiasis in non-human primates, especially in New World monkeys,

indicate that these nematodes may cause profound oral inflammation and irritation, particularly in times of high parasite exposure, and predispose the affected animals to fatal secondary infections.[1,4]

AFIP Diagnosis: Oral mucosa, multiple sites: Intraepithelial adult spirurids, with multifocal minimal lymphocytic inflammation, etiology consistent with *Gongylonema* sp., Goeldi's monkey (*Callimico goeldii*), primate.

Conference Comment: There is significant slide variation with some slides having a section of pancreas and attached duodenum with intraluminal and submucosal nematode parasites.

As mentioned by the contributor, the key histologic features of spirurids include a characteristic small, usually thick-shelled, embryonated egg; cuticular ornamentations around the buccal cavity; coelomyarian musculature; uninucleate multicellular intestine often lined by microvilli that form a brush border; and, lateral chords that may be quite large. In some sections, the spirurids within the oral mucosa have cuticular bosses and lateral alae. The cuticular bosses are characteristic for *Gongylonema* sp. and are located on the anterior end of the parasite. Grossly, *Gongylonema* sp. are long thin worms that often form a zigzag pattern in the mucosa and submucosa and appear "stitched" into the tissue.[5]

Some sections contain pancreas and duodenum with intraluminal and submucosal spirurids. These spirurids are *Pterygodermatites* sp., which have characteristic lateral alae in a sublateral position on the anterior end of the parasite. Adult parasites may be found in the lumen of the small intestine with their anterior ends embedded in the mucosa. The larvae, when present, are deeper in the submucosa.[6]

Other intraepithelial parasites include: *Capillaria* sp., *Anatrichosoma* sp., and *Trichosomoides* sp.

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