CASE I – N0803234 (AFIP 3102615).

Signalment: Juvenile, female Northern elephant seal, Mirounga angustirostris

History: The seal was found stranded in California. On physical exam, it was found to be blind and had bilateral cataracts. It spent 8 months in a stranding center and was later sent to Adventure Aquarium in Camden, NJ. The seal did well, but a week after arrival it was found floating and unresponsive following administration of 4 tabs (“large dog size”) of Drontal. The seal was known to be Toxoplasma gondii positive.

Gross Pathology: At necropsy, the animal had moderately decreased subcutaneous blubber thickness. The haircoat was extremely sparse and completely absent over much of the animal. There were numerous multifocal to coalescing cutaneous ulcers and erosions along the ventrum extending from the muzzle to the anus and on the ventral aspects of the fore and hind flippers. Both eyes had opaque, cataractous lenses. The teeth were covered with moderate to abundant dental calculus. A vascular anomaly involving the portal vein and caudal vena cava was identified.

Laboratory results: Immunohistochemistry: Toxoplasma gondii antibody applied to sections of brain revealed strong positive staining of bradyzoite cysts for Toxoplasma gondii antigen (Fig. 1-3). No definitive staining of cysts or tachyzoites was seen in the skin lesions.

Histopathologic Description: Within the leptomeninges and surrounding blood vessels throughout the cortex, cerebellum and brain stem, there are multifocal aggregates of lymphocytes, plasma cells and histiocytes (Fig. 1-1). The surrounding parenchyma is rarefied and gliotic with neuronal chromatolysis and necrosis. Within some inflammatory foci are thin-walled tissue cysts up to 40 x 60 um, that contain numerous 1-2 um elongate bradyzoites consistent with T. gondii (Fig. 1-2). A few necrotic foci with moderate lymphohistiocytic inflammation and associated tissue cysts are observed within sections of skeletal muscle. Individual tissue cysts without associated inflammation or necrosis are present in the ovary and in the wall of a medium sized myocardial artery.

Contributor’s Morphologic Diagnosis: Brain: meningoencephalitis, necrotizing and
1-1. Meninges, elephant seal. The meninges are mildly expanded by a cellular infiltrate that occasionally extends into the underlying cerebrum. (HE 200x).

Photomicrograph courtesy of University of Pennsylvania, School of Veterinary Medicine, Laboratory of Pathology and Toxicology.

lymphohistiocytic, multifocal, moderate to severe with intranisional protozoal cysts consistent with T. gondii.

Contributor’s Comment: Toxoplasma gondii is a coccidian parasite that is found throughout the world and infects an extensive range of intermediate hosts in which it causes both clinical and more commonly, subclinical disease.(7) Domestic and wild felids are the only known definitive hosts and also serve as intermediate hosts.

Infection occurs by ingestion of oocytes excreted in the feces of felids, by ingestion of tissues of intermediate hosts that contain bradyzoites or tachyzoites, and less frequently by vertical transmission. Once ingested, sporozoites excyst and multiply in the intestinal epithelial cells as tachyzoites. Tachyzoites can either disseminate and infect cells throughout the body resulting in necrosis and non-suppurative inflammation characteristic of toxoplasmosis, or encyst in tissues as bradyzoites. Follo wing ingestion of tissue cysts by an intermediate host, bradyzoites will excyst, become tachyzoites, and the cycle continues.(2,6,7)

There is one report of toxoplasmosis in an elephant seal pup.(4) Micoscopic lesions included multifocal nonsuppurative meningocerebralitis and multifocal tissue cysts with and without associated inflammation in the cerebrum. Cyst morphology was consistent with T. gondii, and protozoal stain ed positively with T. gondii, but not with N. caninum, polyclonal antibody. Focal lymphoplasmacytic inflammation was present in the brain, retina, optic nerve, and renal tubules, and non-suppurative glossitis with necrosis and ulceration was also observed.(4)

Toxoplasmosis in marine mammals has recently become of particular concern since being identified as a leading cause of encephalitis and death in the threatened Southern sea otter (Enhydra lutris nereis).(8) Since 1951, toxoplasmosis has been reported in various species of seals, dolphins, sea lions, a West Indian manatee and a beluga whale.(5) Serological assays of numerous species of marine mammals suggest common and widespread exposure. (5)

It is unclear how marine mammals become infected with T. gondii as they rarely consume recognized intermediate hosts, and T. gondii is not known to parasitize fish or invertebrates. It has been proposed that infection occurs through consumption of oocysts that are present in the marine environment via surface runoff or municipal sewage contaminated by cat feces. (9,11) In support of this theory, T. gondii oocysts have been shown to sporulate and survive in seawater for several months. (9) Laboratory experiments have shown that bivalves can concentrate T. gondii oocysts and recent wildlife studies have confirmed this for T. gondii in species such as mussel, funeate seastar, and others.(9) Invertebrate filter feeders can serve as a source of infection for marine mammals.

Additionally, a type X strain of T. gondii that has recently been isolated from over 72% of all sea otter infections(2) was identified in the California mussel as well as in several coastal dwelling felids and canids.(10)

AFIP Diagnosis: Cerebrum, brainstem: Meningoencephalitis, necrotizing, histiocytic, multifocal, mild with lymphoplasmacytic peri vascular cuffing and multiple tissue cysts consistent with T. gondii.

Conference Comment: Toxoplasma gondii is a ubiquitous organism that is indiscriminant in nature, infecting all warm-blooded animals, but members of the family Felidae are the only known definitive hosts. (6) Domestic and wild felids are the only known definitive hosts and also serve as intermediate hosts.

Systemic disease occurs mostly in young or immunocompromised animals, but members of the family Felidae are the only known definitive hosts. (6) Systemic disease occurs mostly in young or immunocompromised animals, but members of the family Felidae are the only known definitive hosts. (6)
movement to adjacent cells with thin tetracyclic organ resulting in the characteristic necrotizing lesion often seen with toxoplasmosis. Cell-mediated immunity seems to be the more important than humoral immunity, and over time animals develop a quiescent infection characterized by cysts with thin outer wall containing numerous bradyzoites, which are more slender and less susceptible to destruction by proteolytic enzymes than tachyzoites. (1)

Numerous organ systems are affected by toxoplasmosis, with pulmonary lesions and central nervous system lesions having the highest prevalence. (1) Within the lung, lesions are characterized by necrosis of alveolar walls, bronchiolar epithelium, and the vasculature with accompanying interstitial pneumonia with mononuclear cell invasion into the alveolar walls. (1) Multifocal necrosis within the central nervous system and accompanying non-suppurative inflammation can occur with toxoplasmosis. Microglial nodules are occasionally seen with chronicity within the parenchyma of the central nervous system. (1)

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http://www.vet.upenn.edu/departments/pathobiology/pathology

References:


**CASE II – PA 4596 (AFIP 3103740).**

**Signalment:** Adult, male (*Macaca fascicularis*)

**Cynomolgus macaque**

**History:** This animal had been experimentally infected with *Mycobacterium tuberculosis* 8 weeks previously and was being sacrificed as an acute control. The lesions submitted were incidental necropsy findings.

**Gross Pathology:** In the transverse and ascending colon, extending into the cecum, approximately one dozen, slender, thread-like parasites suggestive of Nematodes were noted (Fig. 2-1). These ranged from 6-12 mm in length depending on their state of extension and were very darkly colored. Additionally, present primarily within the cecum were numerous circumscribed somatic nodules of submucosal darkening (Figs. 2-2 and 2-3). Transection across several of these structures revealed cavitary areas 2-3 mm in diameter, containing small amounts of thin, dark brown fluid.

**Histopathologic Description:** Slides from multiple blocks are submitted, but are similar in appearance. Present within the submucosa are somewhat circumscribed cavitory lesions filled with a combination of necrotic debris and abundant mixed inflammatory cells, including large numbers of epithelioid macrophages, multinucleated giant cells and more peripheral lymphoplasmacytic infiltrates. Also noted centrally within these submucosal nodules are numerous metazoan parasite structures identifiable as Nematodes based on the presence of an external cuticle, musculature, digestive and reproductive tracts (the latter not visible in all sections submitted) (Fig. 2-4, 2-5, 2-6).

2-1. Colon, Cynomolgus macaque. Oesophagostomum nematodes.


2-3. Intestinal serosa, Cynomolgus macaque. Serosal granulomatous nodules suggestive of previous infection.

Gross photographs courtesy of the Division of Laboratory Animal Resources, University of Pittsburgh, Pittsburgh, Pennsylvania
Contributor’s Comment: The worms present were subsequently identified by a parasitologist (DB) as *Oesophagostomum* sp. (with species identification pending). Slide mounted specimens measured 8.0 to 13.4 mm in length and possessed morphologic characteristics consistent for the subfamily Oesophagostominae with in the family Strongylidae. Generic assignment to *Oesophagostomum* is based on specimens having a well defined perioral corona radiata; a straight forwardly directed mouth possessing a collar with two lateral and iron pigment sometimes visible within intestinal cells.

Contributor’s Morphologic Diagnosis: Typhlitis/colitis, submucosal, necrotizing and granulomatous, subacute, with numerous metazoan parasites consistent with Strongyle-type nematodes.

Further histological characteristics present allow identification as Strongyles, including the presence of platmyarian musculature, prominent vacuolated lateral chords and characteristic intestinal tract with brush borders and iron pigment sometimes visible within intestinal cells.
four submedial cephalic papillae, and a deep posterior annular constriction; a transverse cervical ventral groove that extends around the body towards the dorsal side; and a dilation of the cuticle between the mouth collar and cervical ventral groove. Two leaf crowns were present; a shallow cylindrical buccal capsule; and an esophageal funnel possessing lancets. Males possessed a complex bursa with rays consistent with those described for the genera, spicules of equal length, and a gubernaculum. Females had parallel uterine branches and a tail that tapered to a point, possessing a vulvar opening positioned slightly anterior to the anus.

The oesophagostomes, sometimes referred to as nodular worms, are among the most common and injurious parasites of monkeys and apes. Worms characteristically produce nodules or cysts in the submucosa or muscularis of the large intestine and less frequently in ectopic sites. Although confusion exists about species identification, *apiostomum*, *bifurcum*, *aculeatum* and *stephanostomum* are recognized in the genus Oesophagostomum.

Adult worms live in the lumen of the bowel in their definitive host. Eggs are passed in the feces, hatch and release larvae that mot twice to become infective. Third stage larvae when swallowed by a new host, burrow into the submucosa of the small or large intestine, molt again to fourth stage larvae and return to the lumen of the large intestine, where they molt again to become mature worms.

Seen not uncommonly in baboons, mangabeys, macaques and great apes, infestation in New World monkeys is rare. Prior to the influx of feral, recently imported Chinese macaques in recent years, the chronic, healed lesions from these parasites were occasionally recognized as discrete and circumscribed, highly mineralized nodules visible on the serosal margin of the bowel (Fig. 2-3). Such lesions generally did not denote histologic evidence of recognizable parasites. The submitted case demonstrates an active nonhuman primate infection.

Oesophagostomum infestation from a variety of species is of course well recognized in numerous other animal species including pigs (*O. dentatum*), cattle (*O. radiatum*), sheep (*O. columbianus*), and several wild ruminants—in which such “nodular worm” disease may be associated with significant morbidity and mortality.

**Brief review of the major features of nematodes in histologic section.**

**Cuticle:** The cuticle is the outermost covering of a nematode, which can range in thickness from being very prominent to almost imperceivable. Alae, which are winglike extensions of the cuticle, can also be used to identify certain nematodes.

**Hypodermis:** The hypodermis is immediately internal to the cuticle and extends into the body cavity, or pseudocoelom. Projections of the hypodermis into the pseudocoelom are called lateral chords. These chords can have many different shapes and are helpful in parasite identification.

**Musculature:** Muscle cells extend from the hypodermis into the pseudocoelom and are composed of a contractile element and a cytoplasmic element. On a normal H&E slide, the cytoplasmic portion is usually clear, and the contractile portion is bright pink to red. Muscles are categorized as being either coelomyarian or platymyarian. Coelomyarian muscles extend into the body cavity in a circular manner, whereas platymyarian muscles are often flattened against the hypodermis and do not extend into the body cavity. Coelomyarian muscles are often numerous and with many being present in a single section of a nematode, and this explains the second portion of the muscle naming nomenclature, polymyarian (e.g., coelomyarian–polymyarian musculature). Platymyarian cells usually extend along the length of the worm and are few in number, and their arrangement is described as meromyarian.

**Digestive Tract:** Nematodes have a digestive tract composed of the following structures: a mouth, buccal cavity, esophagus, intestine, and anus. The digestive tract size is described relative to the diameter of the nematode, and thus the descriptors large, medium, and small are used. The number of cells lining the intestine are commonly described as either ‘few multinucleate’ cells or ‘many uninucleate’ cells. Often the intestinal cells contain pigment from digested blood or bile, and this can also be helpful when present to identify them as intestinal cells.
Human Oesophagostomiasis is an infrequently described and recognized parasite infection in humans, generally caused by *Oesophagostomum bifurcum*. It is a regional and very localized problem in Africa, but is considered common in northern Togo and Ghana. Human infestation may cause localized abdominal pain and discomfort, commonly in the right lower quadrant and is often accompanied by epigastric or periumbilical masses.

**AFIP Diagnosis:** Colon: Granulomas, multifocal, with few st rongyloid nematodes, *Macaca fascicularis*, primate.

**Conference Comment:** There is considerable slide variation; some sections contained coal scarring granulomatous inflammation centered on the nematodes but not forming distinct granulomas.

The contributor did a magnificent job describing not only the identification features and life cycle of this nematode parasite, but also gave an excellent summary of comparative pathology.

For the pathologist, it is important to systematically describe nematode parasites in tissue sections. One satisfactory method is to start at the outer layers and work one's way in. A brief review of the major histologic identifiable features is presented here and is based on Dr. Chris Gardiner's guidelines in *An Atlas of Metazoan Parasites in Animals Tissues*. (4)

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**References:**

Abdominocentesis fluid protein <2.5 g/dl.

**Histopathologic Description:**
Kidney and liver. The parenchyma of both the kidney and liver is invaded by a well-demarcated, partially encapsulated, expansile and infiltrative neoplasm consisting of haphazardly arranged and densely packed sheets of polygonal to spindle-shaped (blastemal and mesenchymal) cells, an d less commonly, groups of cuboidal to columnar (epithelial) cells that form incomplete tubular structures (Fig. 3-1). Sheets of cells are encapsulated by a fibrous capsule or compressed residual stroma of the kidney and are subdivided by variably thick bands of connective tissue. Cells of the blastemal component are polygonal, have indistinct cell borders, scant, pale, eosinophilic cytoplasm, and a round, hyperchromatic nucleus. The blastemal component blends with spindle-shaped (mesenchymal) cells separated by scant to moderate amounts of fibrillar, eosinophilic (collagenous) extracellular matrix. A loose, myxoid, extracellular matrix is present between spindle-shaped cells in some areas. Less commonly and usually located adjacent to collagenous stroma are cuboidal to columnar cells that form tubular structures with indistinct lumens. The cells have scant eosinophilic cytoplasm and are often basally located nuclei. Blasto and mesenchymal cells are strongly immunopositive for vimentin and are cytoplasmic-negative. Approximately 40% of the spindle-shaped cells are immunopositive for desmin and all of the spindle-shaped cells are immunonegative for smooth muscle actin. The cells in the trabeculae of connective tissue between sheets of cells are faintly immunopositive for smooth muscle actin. Trichrome staining demonstrates scant collagen within the sheets of spindle-shaped cells and abundant collagen in the trabeculae between sheets of cells. The cuboidal cells forming tubules and some groups of less organized, polygonal cells are strongly immunopositive for cytokeratin and negative or faintly positive for vimentin. Staining with periodic acid-Schiff demonstrates a scant, discontinuous basement membrane subjacent to some tubular structures. Mitotic figures are 8-9 per 400X field among the blastemal/mesenchymal component. Anisokaryosis is prominent. The adjacent renal parenchyma is atrophic, with widespread loss of tubules and glomeruli and collapse of the interstitium. The hepatic parenchyma is atrophic with loss of hepatocytes and collapse of portal regions adjacent to the neoplasm.

**Contributor’s Morphologic Diagnosis:** Malignant nephroblastoma, kidney and liver.

**Contributor’s Comment:** Nephroblastomas (also called “embryonal nephromas” in older literature and Wilms’ tumor in humans) are theorized to arise from rests of metanephric blastema and usually develop in young animals and children.(1,7,8) Nephroblastomas are rare in horses and most other animal species, except for chickens and swine.(6,12,2,10,5,11,4) The gross and histologic features of nephroblastoma in the horse are rarely described.(6) Nephroblastomas are occasionally diagnosed in adult animals, as in the presented case in a 5-year-old horse.(5,11,4) Nephroblastomas represent defective nephrogenesis and...
Historically, nephroblastomas have been categorized according to the relative amounts of each of the three cellular components, with a "triphasic nephroblastoma" containing approximately equal amounts of each of the three cell lineages. In the neoplasm presented here, the epithelial component was present multifocally, but not to the lung or more distant regions of the liver, suggesting coelomic metastasis.

The genetic pathology that results in Wilms' tumor in children appears to be complex, and, in some cases, the development of Wilms' tumor in children is associated with other congenital malformations. The protein product of the Wilms' tumor suppressor gene-1 (WT-1) is a zinc-finger DNA binding protein and an essential regulator of renal development. Inactivation of the WT1 gene is documented in a small number of Wilms' tumors in children and is believed to prevent the differentiation of primitive metanephric cells. The remaining Wilms' tumors in human beings are assumed to be due to defects in other genes, including WT3 and others. Genetic analysis was not performed on tissue from this case.

The typical hallmark histologic features of the nephroblastomas are loosely arranged spindle cells with variable amounts of mesenchymal and epithelial elements. The neoplasm has been found in rats exposed to different tumor producing agents. Metastasis in canine tumors occurred in over 50% of the reported cases, whereas in pigs and calves metastasis is uncommon. In dogs, particularly in German Shepherds, these tumors can form extramedullary, in tradural spinal masses usually accompanied by multifocal bone lesions.

In the sections examined during conference, the neoplasm contained all three elements required for the diagnosis of a nephroblastoma: blastemal, mesenchymal, and epithelial, although not evenly represented in the neoplasm presented here. Immunohistochemical staining of the tissues from this case confirmed the coexistence of mesenchymal and epithelial components within sheets of embryonic cells. Myofibroblastic differentiation was demonstrated by vimentin and desmin immunopositivity. Cells forming tubular structures or located adjacent to trabeculae often were immunopositive for cytokeratin. Other samples of this neoplasm from the kidney, pancreas, and liver contain more of the epithelial component, consisting primarily of tubular structures; rudimentary glomeruli were not identified in examined sections from this case. The neoplasm presented here extended to anatomic structures adjacent to the right kidney, but not to the lung or more distant regions of the liver, suggesting coelomic metastasis.

In human beings, nephroblastomas that have cytologic features of anaplasia, including enlarged nuclei, hyperchromasia of nuclei, and enlarged, multinucleated, mitotic figures, are designated as having an unfavorable histology and are treated with different protocols. The neoplasm has been found in rats exposed to different tumor producing agents. Metastasis in canine tumors occurred in over 50% of the reported cases, whereas in pigs and calves metastasis is uncommon. In dogs, particularly in German Shepherds, these tumors can form extramedullary, in tradural spinal masses usually accompanied by multifocal bone lesions.
CASE IV - 03-8246 (AFIP 3102495).

**Signalment:** 4-month-old pig

**History:** This pig was submitted with a history of sudden death.

**Gross Pathology:** There was a generalized serofibrinous pleuritis and multiple widespread foci of fibrinous pneumonia in both lungs. Regional lymph nodes were increased in size and hemorrhagic. Small white foci surrounded by a hyperemic zone were disseminated in the skin.

**Laboratory results:** *Actinobacillus suis* was isolated from the pleura, lung, skin and other organs.

**Histopathologic Description:** In the lung section submitted, there is a sero fibrinous pneumonia with many necrotic leukocytes (Fig. 4-1, 4-2). These lesions were multifocal and generalized in both lungs. The necrotic leukocytes appear as round cells with pyknotic nuclei and cells with a streaming of pale basophilic chromatin, the so-called “oat cells.” Small cocobacilli (gram-negative) are present in the alveolar exudate, and few bacterial emboi are present in some sections. Severe capillaries are thrombosed. There is a severe fibrinous pleuritis with necrotic leukocytes similar to those in the lung lesions.

**Contributor’s Morphologic Diagnosis:** Severe acute fibrinoluekocytic pleuroneumonia with many “oat cells” and the presence of intralezional cocobacilli.

**Contributor’s Comment:** The skin lesions observed grossly were characterized by small dermal vessels thrombosed and/or occluded by bacterial emboli (small bacterial emboli (small 7. Khoury JD: Nephroblastic neoplasms. Clin Lab Med 25(2):341-361, 2005.
gram-negative co coccobacilli). Th ey were in filtrated an d surrounded by in flammatory cells, m ainly n ecrotic leukocytes similar to those in the lung. Small coccobacilli were also present in the inflammatory infiltrates.

The multifocal and widespread pneumonia, and the skin lesions observed in th is pig are co mpatible with a septicemia caused by *Actinobacillus suis*. Clinical cases of *A. suis* o ccur more freq uently in h igh-health-status herds(6). T he m ost common manifestation of t he infection is septicemia and sudden death in su cking and recently wea ned pigs(6). A disease resem bling pleuropneumonia cause d by *A. pleuropneumonia*, and skin lesion s si milar to those in the lung. Small coccobacilli were also present in the inflammatory infiltrates.

The pneumatic lesions caused by *A. suis* can have two patterns. On e of t hem is a fo cal lo cally ex tense fibrino hematogenic, fibrinoleukocytic and n ecrotizing pneumonia or pleuropneumonia affecting t he m iddle or the caudal l ung lob es, which m ay b e unilat eral or bilateral(2). Th ese lesions are v ery simi lar to tho se caused by *A. pl europneumonia*, and are proba bly originating from an ai rborne ent ry of the organism(2). T he o ther pattern is a g ener alized multifocal pneumonia indicating h ematogenous ori gin. T his multifocal widespread pneumonia is a com mon finding in cases of *A. suis* septicemia. Other lesions observed in septicemic cases are petechial hemorrhages i n the organs, multifocal necrosis and inflammation in the liver, spleen, kidney an d skin, splenomegaly, ser ofibrinous pericarditis, pleuritis an d p eyritis, p olyarthritis, v alvular endocarditis, and rhomboid sk in lesions similar t o those observed in cases of erysipelas(6).

The fibrinous pneumonia with many necrotic leukocytes appearing as “oat cells” is c haracteristic of *A. pleuropneumonia* a nd *A. suis* in s wine (2). Different serotypes of *A. pl europneumonia* p roduce R TX-toxins (ApxI, II a nd II) w hich a re cy totoxic for t he porcine neutrophils an d m acrophages (2, 4). S ome strains of *A. suis* produce a RTX -toxin (A px I) (6). “Oat cells” are also present i n t he fibrous pneumonia cause d b y *Mannheimia h aemolytica* in cattle, sh eep an d goat (2).

**AFIP Diagnosis:** L ung: Pneumonia, necrotizing, histiocytic and n ecrotic, fibrin, diffuse interstitial and alveolar edema, and numerous colonies of coccobacilli, pig, porcine.

**Conference Comment:** *Actinobacillus suis* is a gram negative, nonmotile, n onencapsulated aero bic an d facultative anaerob ic co cobbacillus t hat is often an inhabitant of t he o ral cavity and upper r espiratory t ract of pigs of any age and the vagina of clinically healthy sows. (6) *A. suis* ca n cause rhomboid skin lesions secondary to vasculitis, and d th is m anifestation can b e co nfused with erysipelas. Petechial to ecchymotic hemorrhages can occur i n m ultiple organ s to in clude th e lu ng, kidney, heart, liv er, sp leen, an d i ntestines. T hese lesions a re often m ost o ccurred in t he l ung s w ith a s triking resemblance t o t he h ish of pleuropneumonia. In s ow s, *A. suis* ca n cause m etritis, men ingitis, an d a bort i on. Histologically, bact erial t hromboemboli randomly scattered in the vasculature of t he previously mentioned organs is suggestive of *A. suis*. (6)
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References: