Conference 1
8 September 2008

Conference Coordinator:
Todd M. Bell, DVM,

Wednesday Slide Conference

Moderator:

Todd Johnson, DVM, Diplomate ACVP

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.

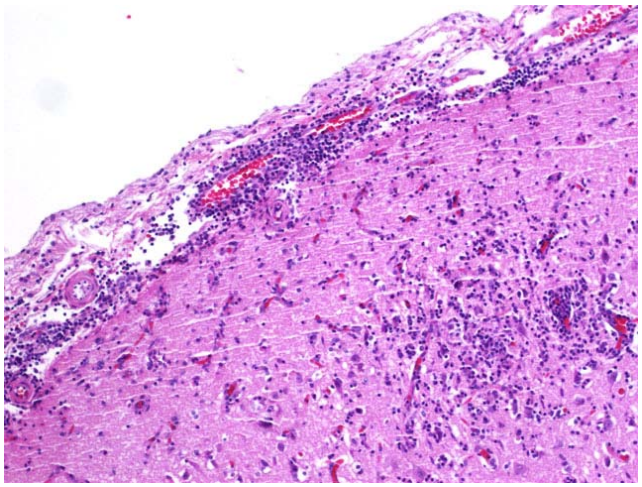
Gross Pathology: At necropsy, the animal had moderately decreased subcutaneous blubber thickness. The haircoat was extremely sparse and completely absent over much of the animal. There were numerous multifocal to coalescing cutaneous ulcers and erosions along the ventrum extending from the muzzle to 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

* Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists and the C. L. Davis Foundation.



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 intralesional 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 sporulated oocysts excreted in the feces of felids, by ingestion of tissues of intermediate hosts that contain encysted 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 the necrosis and non-suppurative inflammation characteristic of toxoplasmosis, or encyst in tissues as bradyzoites. Following ingestion of tissue cysts by an intermediate host, bradyzoites will excyst, become tachyzoites, and the cycle continues.(2,6,7)

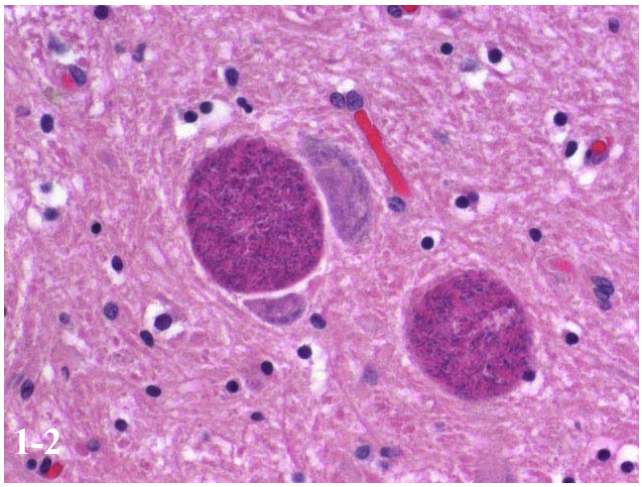
There is one report of toxoplasmosis in an elephant seal pup. (4) Microscopic lesions included multifocal non-suppurative meningoencephalitis and multiple tissue cysts with and without associated inflammation in the cerebrum. Cyst morphology was consistent with *T. gondii,* and protozoa stained positively 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)

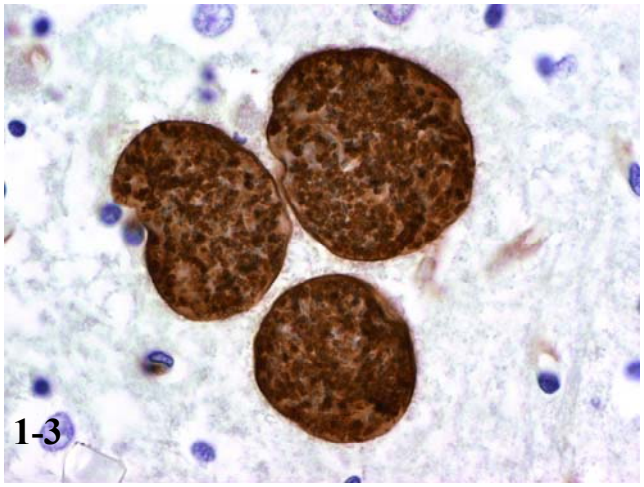
Toxoplasmosis in marine mammals has recently become of particular concern since being identified as a leading cause of encephalitis and death in the threatened Southern sea otter (*Enhydra lutris nereis*).(8) Since 1951, toxoplasmosis has been reported in various species of seals, dolphins, a sea lion, a West Indian manatee and a beluga whale.(5) Serological assays of numerous species of marine mammals suggests 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 enter the marine environment via surface run-off 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 sea water for several months. (9) Laboratory experiments have shown that bivalves can concentrate *T. gondii* oocysts (9) and recently, a wild California mussel was confirmed positive for *T.* gondii (10) suggesting that 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 in 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 perivascular cuffing and few protozoal cysts, Northern elephant seal (*Mirounga angustirostris)*, pinniped.

Conference Comment: *Toxoplasma gondii* is a ubiquitous organism that is indiscriminate in nature, infecting all warm-blooded animals, but members of the family Felidae are the only known definitive hosts. (6) Systemic disease occurs mostly in young or immunocompromised animals, and a lack of proper macrophage function in these neonatal animals contributes directly to this outcome. (1) *Toxoplasma* can infect many different cell types, and this leads to rapid dissemination throughout the host. It can infect many different leukocytes to include macrophages, lymphocytes, and granulocytes and be carried in the





1-2. Brainstem, elephant seal. Low numbers of protozoal cysts which contain myriad 2-4 micron bradyzoites. (HE 400X).
1-3. Cerebrum, elephant seal. Protozoal cysts are strongly immunopostive for Toxoplasma gondii. (600X).

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

---

bloodstream or in lymph via a cell carrier or independently in plasma. (1) *Toxoplasma* then enters a host cell by active penetration of the host cell-membrane and can contort itself in multiple ways to achieve entry into the cell. Once in the cell, the tachyzoite changes shape to from a more ovoid structure and is surrounded by a parsitophorous vacuole that protects it from the host's immune response.(7) Tachyzoites multiply with the parasitized cell, eventually killing it, with subsequent movement to adjacent cells within the resident organ resulting in the characteristic necrotizing lesion often seen with toxoplasmosis. Cell mediated immunity seems to be the more important that humoral immunity, and over time animals develop a quiescent infection characterized by cysts with a 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 an 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)

Contributor: University of Pennsylvania, School of Veterinary Medicine, Laboratory of Pathology and Toxicology
http://www.vet.upenn.edu/departments/pathobiology/pathology

References:

1. Brown CC, Dale CB, Barker IK: The Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 270-272. Elsevier Limited, St. Louis, MO, 2007
2. Conrad PA, Miller MA, Kreuder C, James ER, Mazer J, Dabrtiz H, Jessup DA, Gulland F, Grigg ME: Transmission of Toxoplasma: Clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment. Int J Parasitol 35:1155-1168, 2005
3. Dubey JP: Toxoplasmosis-a waterborne zoonosis. Vet Parasitol 126:57-72, 2004
4. Dubey JP, Lipscomb TP, Mense M: Toxoplasmosis in an elephant seal (*Mirounga angustirostris*). J Parasitol 90:410-411, 2004
5. Dubey JP, Zarnke R, Thomas NJ, Wong SK, Van Bonn W, Briggs M, Davis JW, Ewing R, Mense M, Kwok OCH, Romand S, Thulliez P: *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona* and *Sarcocystis canis*-like infections in marine animals. Vet Parasitol 116:275-296, 2003
6. Gardiner CH, Fayer R, Dubey JP: An atlas of protozoan parasites in animal tissues, 2nd ed., pp. 53-56. Armed Forces Institute of Pathology, Washington, DC 1998

7. Hill DE, Chirukandoth S, Dubey JP: Biology and epidemiology of *Toxoplasma gondii* in man and animals. Animal Health Research Reviews 6:41-61, 2005
8. Kreuder C, Miller MA, Jessup DA, Lowenstine LJ, Harris MD, Ames JA, Carpenter TE, Conrad PA, Mazet JAK: Patterns of mortality in Southern sea otters (*Enhydra lutris nereis*) from 1998-2001. J Wildl Dis 39:495-509, 2003
9. Lindsay DS, Collins MV, Mitchell SM, Cole RA, Flick GJ, Wetch CN, Lindquist A, Dubey JP: Sporulation and survival of *Toxoplasma gondii* oocysts in sea water. J Eukaryot Microbiol 50:687-8, 2003
10. Lindsay DS, Collins MV, Mitchell SM, Wetch CN, Rosypal AC, Flick GJ, Zajac AM, Lindquist A, Dubey JP: Survival of *Toxoplasma gondii* oocysts in Eastern oysters (*Crassostrea virgi nica*). J Parasitol 90:1054-1057, 2004
11. Miller MA, Gardner IA, Kreuder C, Paradics DM, Worcester KR, Jessup DA, Dodd E, Harris MD, Ames JA, Packham AE, Conrad PA: Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). Int J Parasitol 33:997-1006, 2002
12. Miller MA, Miller WA, Conrad PA, James ER, Melli AC, Leutenegger CM, Dabritz H A, Packham AE, Paradics DM, Harris M, Ames J, Jessup DA, Worcester K, Griggs M E: Type X *Toxoplasma gondii* in a wild mussel and terrestrial carnivores from coastal California: New linkages between terrestrial mammals, run off and toxoplasmosis of sea otters. Int J Parasitol article in press, 2008

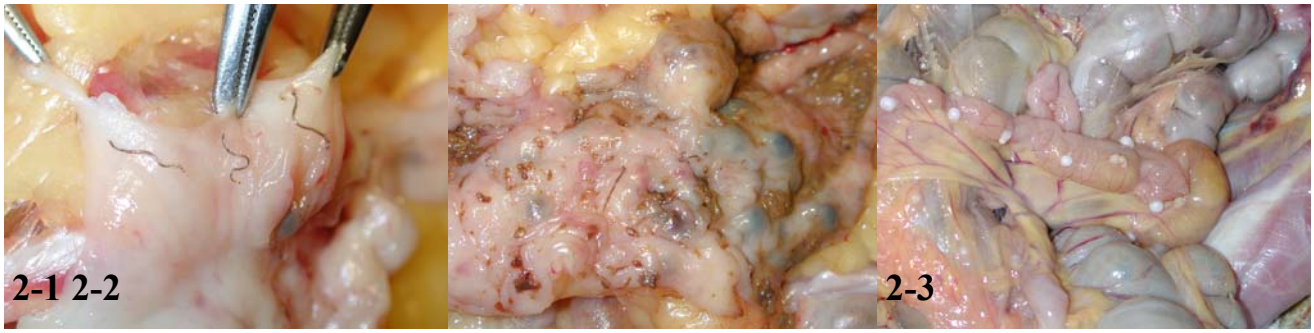
CASE II – PA 4596 (AFIP 3103740).

Signalment: Adult, male (*Macaca fasicularis*) 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.

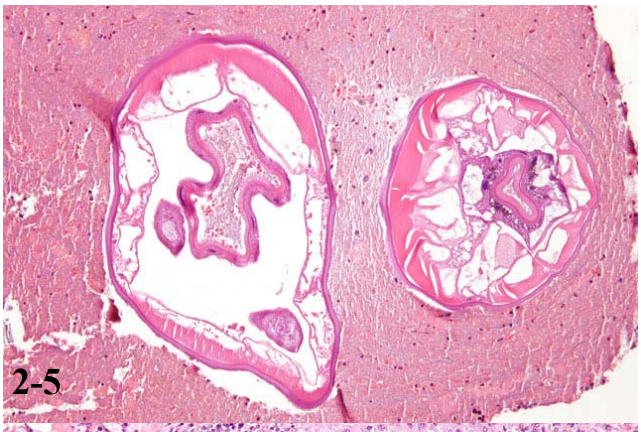
Gross Pathology: In the transverse and ascending colon, extending into the cecum, approximately one dozen, slender, thread-like parasites suggestive of Nematodes were noted (Fig. 2-1). These ranged from 6-12 mm in length depending on their state of extension and were very darkly colored. Additionally, present primarily within the cecum were numerous circumscribed somewhat nodular areas of submucosal darkening (Fig. 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.

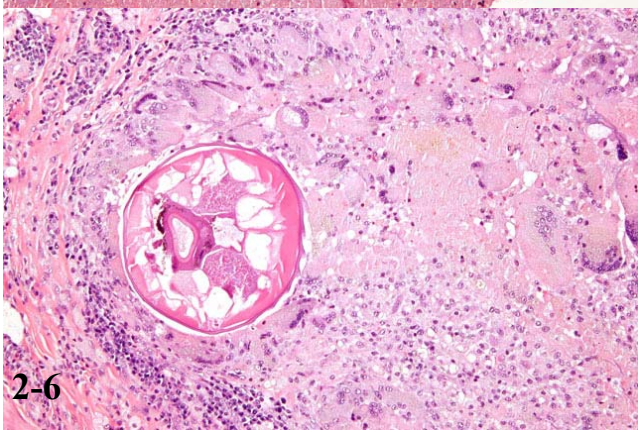
Histopathologic Description: Slides from multiple blocks are submitted, but are similar in appearance. Present within the submucosa are somewhat circumscribed cavitary lesions filled with a combination of necrotic debris and abundant mixed inflammatory cells, including large numbers of epithelioid macrophages, 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.
2-2. Colon, Cynomolgus macaque. Submucosal nodules.
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





2-4. Colon, Cynomolgus macaque (Macaca fascicularis). Granuloma centered on numerous cross sections of nematode larvae. (HE 200X).
2-5. Colon, Cynomolgus macaque (Macaca fascicularis). Nematode larvae are characterized by a smooth cuticle, platymyarian-meromyarian musculature, prominent vacuolated lateral chords and a gastrointestinal tract lined by epithelial cell with a prominent brush border. (HE 100X).
2-6. Colon, Cynomolgus macaque (Macaca fascicularis). Nematode larvae are surrounded by numerous epithelioid macrophages and multinucleated giant cells which are bounded by lymphocytes, plasma cells and a thin fibrous capsule. (HE 200X).

Photomicrographs courtesy of the Division of Laboratory Animal Resources, University of Pittsburgh, Pittsburgh, Pennsylvania

---

Further h istological ch aracteristics p resent allows identification as Strongyles, including the pres ence of platymyarian m usculature, prominent vacuolated lateral chords a nd c haracteristic i ntestinal tract with brush borders a nd iron pi gment som etimes visible wi thin intestinal cells.

Contributor's Mor phologic Diagn osis: Typhlitis/ colitis, su bmucosal, n ecrotizing and granulomatous, subacute, with nu merous m etazoan p arasites con sistent with Strongyle-type nematodes

Contributor's Comment: The worm s present were subsequently id entified b y a parasitologist (DB) as *Oesophagostomum* sp . (with sp ecies id entification pending). Slide mounted specimens measured 8.0 to 13.4 mm in length an d possessed m orphologic characteristics consistent fo r the sub family Oesophagostominae with in the fam ily Strongylidae. Generic assignm ent to *Oesophagostomum* is ba sed on specimens havi ng a well defined perioral co rona ra diata; a straight forwardly directed m outh possessing a co llar with t wo lateral an d

four submedial cephalic papillae, and a deep posterior annular constriction; a transverse cervical ventral groove that extended around the body towards the dorsal side; and a dilation or inflation 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,(9) spicules of equal length, and a gubernaculum. Female's 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.(6) 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.(1)

Adult worms live in the lumen of the bowel in their definitive host. Eggs are passed in the feces, hatch and release larvae that molt 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.(2)

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 demonstrate histologic evidence of residual recognizable parasite structures. 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. (5, 8)

Brief review of the major features of nematodes in histologic section. (4)

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., coelmyarian – 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*.(3) It is a regional and very localized public health problem in Africa, but is considered common in northern Togo and Ghana.(7) Human infestation may cause localized abdominal pain and discomfort, commonly in the right lower quadrant and this is often accompanied by epigastric or periumbilical masses.(2)

AFIP Diagnosis: Colon: Granulomas, multifocal, with few strongyloid nematodes, Cynomolgus macaque (*Macaca fascicularis*), primate.

Conference Comment: There is considerable slide variation; some sections contained coalescing 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 section. One satisfactory method it 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)

Contributor: Division of Laboratory Animal Resources, University of Pittsburgh, Pittsburgh, Pa. 15261, **http://www.oorhs.pitt.edu/research/dlar.html**

References:

1. Benirschke K, Garner FM, Jones TC. Pathology of Laboratory Animals Vol. II, pp 1648-1649. Springer-Verlag, New York NY, 1978
2. Binford CH, Connor DH. Pathology of Tropical and Extraordinary Diseases Vol. I, pp 440-445. Armed Forces Institute of Pathology, Washington D.C., 1976
3. Bogers JJ, Storey PA, Faile G, Hewitt E, Yelifari L, Polderman A, Van Marck EA. Human oesophagostomiasis: a histomorphometric study of 13 new cases in northern Ghana. Virchows Arch 439:21-26, 2001
4. Gardiner CH, Poynton SL: An Atlas of Metazoan Parasites in Animal Tissues, pp. 1-43. Armed Forces Institute of Pathology, Washington, DC, 1999
5. Jones TC, Hunt RD, King NW. Veterinary Pathology Sixth Ed. pp 612-613. William & Wilkins, Baltimore MD, 1997
6. Orihel T C, Sebold. Nematodes of the Bowel and Tissues. In: Pathology of Simian Primates Part II: Infectious and Parasitic Diseases, ed. Fiennes R, pp. 76-99. S. Karger, New York, 1972
7. Polderman AM, Anemana SD, Asigri V. Parasitology Today 15(4): 129-30 Apr 1999
8. Soulsby EJL. Helminths, Arthropods and Protozoa of Domesticated Animals 6th Ed. pp 195-200. Williams and Wilkins Company, Baltimore MD, 1971
9. Yamaguti, S. Systema Helminthum, The Nematodes of Vertebrates Vol. II I, pp. 393-397. Interscience Publishers, New York, NY, 1961

CASE III – 06-42786 (AFIP 3102365).

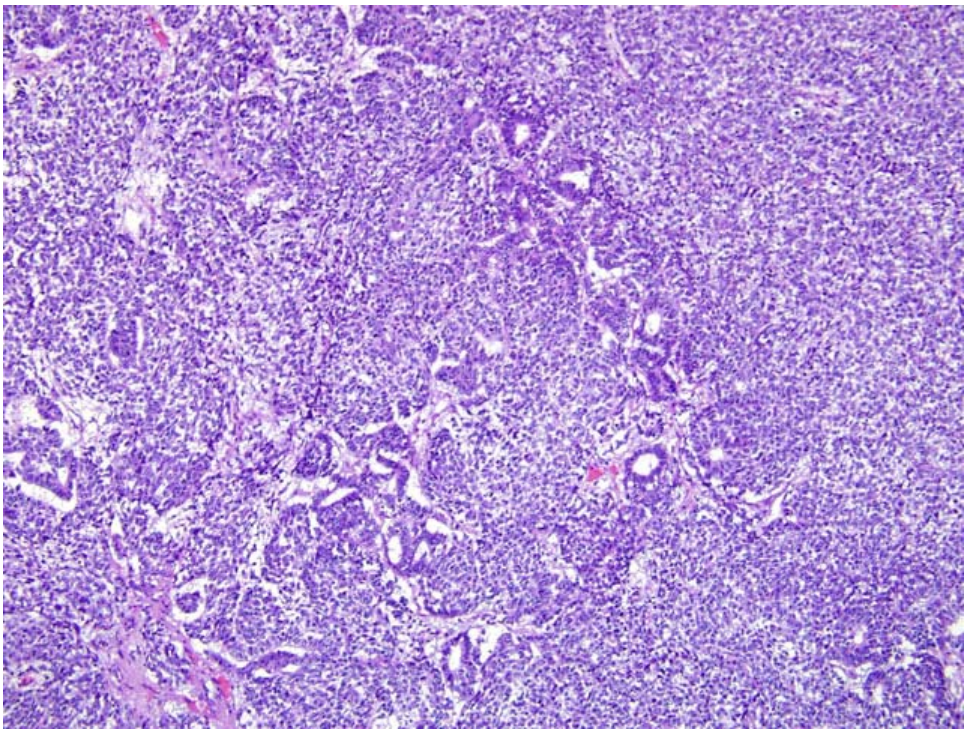
Signalment: 5-year-old, male castrated American quarter horse, *Equus caballus*

History: The horse was used for roping. This horse had moderate, recent weight loss and intermittent reluctance to work on the right hand. The horse was presented for colic that was non-responsive to sedation and anti-inflammatory medications. An abdominal mass and small intestinal distension was diagnosed by palpation and ultrasound. The horse was euthanized due to a poor prognosis.

Gross Pathology: The body is in poor body condition (2/9). In the right anteriodorsal quadrant of the abdomen, a multinodular mass, approximately 60 cm in diameter and weighing ~50 pounds invades and compresses the adjacent organs. The mass is in intimate contact with and invades the parenchyma of the right kidney, liver, and pancreas and extends into the mesentery, causing compression of the duodenum. On sectioning, the mass is firm, solid, and mottled white and pale yellow. The neoplasm effaces approximately half of the parenchyma of the right kidney and extends into the dilated renal pelvis of that kidney. Throughout the abdomen, multiple, firm, white, round nodules, ranging from 0.5 to 5 cm in diameter, are attached to or embedded within the mesentery and omentum. The stomach is distended by gas and approximately 2.5 liters of cloudy, green fluid. The lungs and tracheobronchial lymph nodes are diffusely red-pink, wet, and heavy.

Laboratory Results: 24 mg/dl, creatinine 2.1 mg/dl.

Abdominocentesis fluid: protein <2.5 g/dl.



3-1. Kidney, horse. Nephroblastoma. Effacing normal kidney architecture is a densely cellular neoplasm that occasionally forms variably sized and irregularly shaped tubules. (HE 200X).

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, and, 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 moderately abundant, 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, cuboidal to columnar cells form tubular structures with indistinct lumens. These cells have scant eosinophilic cytoplasm and often a basally located nucleus. Blastemal and mesenchymal cells are strongly immunopositive for vimentin and are cytokeratin-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 Diagnoses: Malignant nephroblastoma, kidney and liver.

Contributor's Comment: Nephroblastomas (also called "embryonal nephromas" in older literature and Wilms' tumor in human beings) 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

their c omponent s ubtypes reflect t he conversion of metanephric mesenchymal cells to ep ithelial stru ctures that occurs d uring ne phrogenesis.(7,8,3) T he ne oplasm presented here contains all three elements required for the diagnosis of a nephroblastoma: blastemal, mesenchymal, and ep ithelial, alth ough no t ev enly rep resented in t he presented section of ki dney and in o ther o rgans. Immunohistochemical st aining of t he t issues from t his case confirm s the coe xistence o f m esenchymal and epithelial co mponents wit hin th e sheets of em bryonic cells. M yofibroblastic di fferentiation was demonstrated by vi mentin an d desmin i mmunopositivity. C ells forming tu bular str uctures or l ocated adjace nt to trabeculae often were immu nopositive for cyto keratin. Other sam ples o f t his neoplasm fr om t he kidney, pancreas an d liv er con tain m ore of th e ep ithelial component, c onsisting primarily of t ubular st ructures; rudimentary gl omeruli were not i dentified in exam ined sections from this case. T he ne oplasm presented here extended to a natomic struct ures adjacent to the right kidney, but not to the lung or more distant regions of the liver, suggesting coelomic metastasis.

Historically, nep hroblastomas have been categorized according to the relative a mount of eac h of t he three cellular com ponents, with a "t riphasic nephroblastoma" containing a pproximately equal am ounts of eac h of t he three cell lin eages.(7,8) In the n eoplasm presented here, cells o f all three d ifferentiation typ es are id entified by cytomorphology and using im munohistochemistry, in varying am ounts am ong di fferent re gions of t he neoplasm. Cells th at do not d emonstrate cytomorphologic f eatures of mesen chymal or epithelial differentiation, i.e., t he blastemal cells, p redominate in this n eoplasm. In human bein gs, nephroblastomas th at have cy tologic feat ures of an aplasia, i ncluding enla rged nuclei, hy perchromasia of n uclei, a nd e nlarged, multipolar m itotic fi gures, are desi gnated as ha ving unfavorable histology i n t he cu rrently use d st aging protocol.(7,9)

The g enetic path ology that resu lts in W ilms' tumor in children a ppears to be com plex, a nd, i n s ome cases, t he development of Wilms' tu mor in ch ildren is associated with other co ngenital m alformations. (1 ,7) Th e protein product of t he Wilms' t umor su ppressor ge ne-1 (WT-1) is a zi nc-finger DNA binding protein a nd an es sential regulator of ren al development. In activation of th e WT1 gene is documented in a sm all number of Wilms' tumors in ch ildren and is believed t o prevent th e differentiation of primitive metan ephric cells. T he remaining Wilms' tumors in human beings are assumed to be due to defects in ot her genes, i ncluding WT3 an d others. Genetic analysis was not performed on tissue from this case.

AFIP Diagn osis: K idney; liver: Nephroblastoma, horse, equine.

Conference Com ment: Nephroblastoma is th e m ost common tumor of the kidney in both the chicken and pig. (8) The se tum ors are less comm on in calves and dogs and apparently very rare in horses, cats, a nd sheep. T his neoplasm has been found i n rat s e xposed t o different tumor p roducing ag ents.(8) Metastasis in can ine tumors occurred i n over 50% of the reported case s, whe reas in pigs a nd cal ves m etastasis is unc ommon. In dogs, particularly in German Shepherds, these tu mors can form extramedullary, in tradural spin al masses usu ally fou nd between spinal cord segments T10 and L2.(8)

The typical hallm ark hist ologic f eatures of t he nephroblastoma are loosel y arrange d spindle cells amongst primitive gl omeruli, ha phazardly a rranged tubules, and densely cellular blastema. (8) Proportions of these elements vary from tumor to tumor and even within regions of t he sam e t umor. C anine nephroblastomas have been sh own to st ain for h uman W ilms t umor gene product C-19.

In the secti ons e xamined during c onference, the blastemal com ponent com promised t he majority of neoplastic cells. Th e ep ithelial co mponent, in cluding rudimentary tubules was p resent multifocally, b ut glomeruloid st ructures w ere not see n. Loose mesenchymal areas were uncommon.

Contributing Institution: Department of Pathobiology, College of Veterinary M edicine, A uburn U niversity, Auburn, Alabama 36849 http://www.vetmed.auburn.edu/index.pl/patho

References:

1. D'Angio GJ: The National Wilms Tumor Study: a 40 year perspective. Lifetime Data Anal 13:463-470, 2007.
2. G oens SD, M oore CM, B rasky KM , et al .: Nephroblastomatosis an d nephroblastoma in nonhuman primates. J Med Primatol 34:165-170, 2005.
3. Grieco V, Riccardi E, Belotti S, Scanzia ni E: Immunohistochemical st udy of porcine ne phroblastoma. J Comp Path 134:143-151, 2006.
4. Headley SA, Saut JPE, Maiorka PC: Nephroblastoma in an adult sheep. Vet Record 159:850-852, 2006.
5. Henry CJ, Tu rnquist SE, Sm ith A, et al.: Primary renal tumors in cats: 19 cases (19 92-1998). J Feline Med Surg 1:165-170, 1999.
6. Jardine JE, Nesbit JW: Trip hasic nephroblastoma in a horse. J Comp Pathol 114:193-198, 1996.

7. Khoury JD: Nephroblastic neoplasms. Clin Lab Med 25(2) 341-361, 2005.
8. Maxie MG, Newman SJ: Nephroblastoma, Urinary system. In: Jubb, Kennedy, and Palmers' Pathology of Domestic Animals, 5th ed., pp. 501-503. Saunders Elsevier, Philadelphia, PA, 2007.
9. Perlman EJ: Pediatric renal tumors: Practical updates for the pathologist. Pediatric Dev Pathol 8: 320-338, 2005.
10. Terrell SP, Platt SR, Chrisman CL, Homer BL, de Lahunta A, Summers BA: Possible intraspinal metastasis of a canine spinal cord nephroblastoma. Vet Pathol 37:94-97, 2000.
11. Yamamoto Y, Yamada M, Nakamura K, et al.: Nephroblastoma with transcoelomic metastasis in a Japanese black bull. J Vet Med Sci 68(8):891-893, 2006.
12. Zoller M, Matz-Rensing K, Fahrion A, Kaup FJ: Malignant nephroblastoma in a common marmoset (*Callithrix jacchus*). Vet Pathol 45:80-84, 2008.

CASE IV - 03-8246 (AFIP 3102495).

Signalment: 4-month-old pig

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

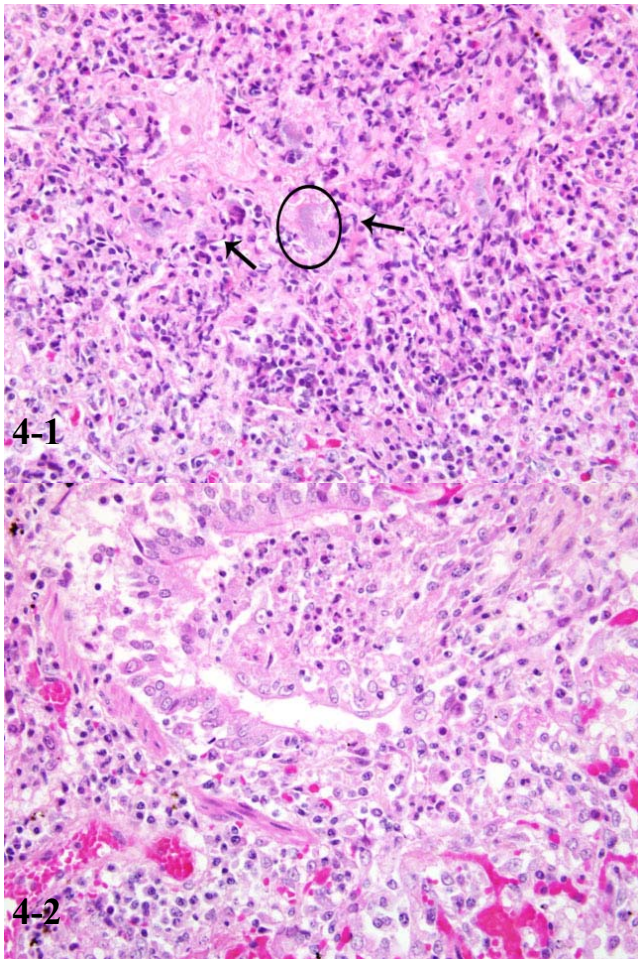
Gross Pathology: There was a generalized serofibrinous pleuritis and multiple widely distributed foci of fibrinous pneumonia in both lungs. Regional lymph nodes were increased in size and hemorrhagic. Small white foci surrounded by a hyperhemic zone were disseminated in the skin.

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

Histopathologic Description: In the lung section submitted, there is a serofibrinous 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 coccobacilli (gram-negative) are present in the alveolar exudate, and few bacterial emboli are present in some sections. Severeal 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 fibrinoleukocytic pleuropneumonia with many "oat cells" and the presence of intralesional coccobacilli.

Contributor's Comment: The skin lesions observed grossly were characterized by small dermal vessels thrombosed and/or occluded by bacterial emboli (small



4.1. Lung, pig. Areas of necrosis admixed with high numbers of alveolar macrophages, fewer lymphocytes and plasma cells and necrotic leukocytes with slender, elongated, streaming nuclei ("oat cells") (arrows) that often surround small colonies of 1-2 um diameter bacilli (circle). (HE 400X)
4-2. Lung, pig. Bronchiolar epithelium is necrotic and replaced by eosinophilic cellular debris admixed with moderate numbers of histiocytes, lymphocytes, fewer plasma cell and rare neutrophils. Bronchiolar lumina often contain exudate composed of cellular and inflammatory debris. (HE 400X).

Common Actinobacillus species in domestic animals.(1,2,3,5)

Actinobacillus pleuropneumonia Pigs		Serofibrinous pleuritis and necrotizing hemorrhagic pneumonia; caudodorsal distribution
Actinobacillus equuli Ho	rses	Common cause of suppurative embolic nephritis in foals
Actinobacillus lignieresii Cattle		Glossitis and stomatitis in cattle (wooden tongue)
Actinobacillus seminis Shee	p	Common cause of bilateral epididymitis in rams

gram-negative co ccobacilli). 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 su is*. Clinical cases of *A. suis* o ccur more frequ ently in h igh-health-status herds(6). T he m ost co mmon manifestation of t he infection is sep ticemia an d su dden death in su ckling and recently wea ned pigs(6). A disease resem bling pleuropneumonia cause d by *A. p leuropneumonia*, and skin lesion s si milar to t hose cau sed b y *Erysipelothrix rhusiopathiae* are reported in older pigs(6).

The pneumonic l esions cau sed by *A. s uis* can have two patterns. On e o f t hem is a fo cal lo cally ex tensive fibrinohemorrhagic, fibrinoleukocytic and n ecrotizing pneumonia or pleuropneumonia affect ing t he m iddle or the cau dal l ung lob es, which m ay b e unilateral or bilateral(2). Th ese lesion s 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 t he organism(2). The o ther pattern is a g eneralized m ultifocal pn eumonia indicating h ematogenous ori gin. T his multifocal widespread pneumonia i s a c ommon fi nding i n ca ses of *A. s uis* septicemia. Oth er lesi ons ob served i n sep ticemic cases are petechial hem orrhages i n se rosa an d ot her organs, multifocal necrosis and inflammation in the liver, spleen, kidney an d ski n, splenomegaly, ser ofibrinous pericarditis, pleuritis an d p eritonitis, p olyarthritis, valvular end ocarditis, and rho mboid sk in lesio ns sim ilar to those observed in cases of erysipelas(6).

The fibrinous pneumonia wi th many necr otic l eukocytes appearing as "oat cells" is c haracteristic of *A. pleuropneumonia* a nd *A. s uis* in s wine (2). Different serotypes of *A. pl europneumonia* p roduce R TX-toxins (ApxI, II a nd I II) w hich a re cy totoxic for t he porcine neutrophils an d m acrophages (2 , 4). S ome st rains o f *A. suis* pr oduce a RTX -toxin (A px I) (6). " Oat cells" are also p resent i n t he fi brinous pneumonia cause d b y *Mannheimia h aemolytica* in cattle, sh eep an d goat (2). All sero types of *M. haemol ytica* p roduce a l eukotoxin being a m ember of t he R TX fam ily of ba cterial t oxins (2). The se necrotic le ukocytes appea ring a s "oat cells" are also prese nt in t he infla mmatory lesions of other organs in cases of *A. Suis* septicemia.

AFIP Diagn osis: L ung: Pne umonia, necr otizing, histiocytic and n eutrophilic, multifocal, mark ed, with vasculitis, necrotic leukocytes ("oat cells"), fibrin, diffuse interstitial and alveolar edema, and numerous colonies of coccobacilli, pig, porcine.

Conference Comment: *Actinobacillus su is* is a gram negative, nonmotile, n onencapsulated aero bic an d facultative anaerob ic co ccobacillus t hat is often an inhabitant of t he t onsils and upp er r espiratory tr act of pigs of any age and the vagina of clinically healthy sows. (6) *A. suis* can cause rhomboid skin lesions secondary to vasculitis, an d th is m anifestation can b e co nfused with erysipelas. Petechial to ecchymotic hem orrhages 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 pr onounced i n the l ungs with a striking resemblance t o t hose of pleuropneumonia. In s ows, *A. suis* can cause m etritis, men ingitis, an d abo rtion. Histologically, bact erial t hromboemboli randomly scattered i n t he vasc ulature of t he p reviously mentioned organs is suggestive of *A. suis.*(6)

Contributor: Department of Pathology and Microbiology, Faculty of Veterinary Medicine,University of Montreal, C.P. 5000, Saint-Hyacinthe, P. Quebec, Canada J2S 7C6, http://www.medvet.umontreal.ca

References:

1. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 96-97. Elsevier Limited, St. Louis, MO, 2007
2. Caswell JL, Williams KJ: Respiratory system. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2 pp. 587-589, 601-606. Elsevier Limited, St. Louis, MO 2007
3. Foster RA, Ladds PW: Male genital system. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3 pg. 590. Elsevier Limited, St. Louis, MO 2007
4. Gottschalk M, Taylor DJ: *Actinobacillus pleuropneumonia*. In: Diseases of Swine, ed. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, 9th ed., pp. 563-576. Blackwell Publishing, 2006.
5. Maxie MG, Newman SJ: Urinary system. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pg. 480. Elsevier Limited, St. Louis, MO, 2007
6. Taylor DJ: *Actinobacillus suis*. In: Diseases of Swine, ed. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, 9th ed., pp. 827-829. Blackwell Publishing, 2006.