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Washington, DC 20306-6000

CASE I – A01-41449 (AFIP 2784703)

Signalment: 4-day-old quarter horse filly, Equine, *Equus caballus*

History: This foal was born 2-weeks premature and was very weak and unable to stand unassisted. The foal was treated with antibiotics and given supportive care for four days. On day four, severe dyspnea developed and the foal was euthanized.

Gross Pathology: The foal was emaciated and the abdominal cavity was filled with urine and there was fibrinous peritonitis. The urachus was swollen, ruptured and covered with fibrin. There was hydrothorax and the lungs were dark purple and diffusely consolidated. Joint fluid was increased, yellow and viscous and there was blood in the left carpal joint. The cerebral meninges were cloudy. Numerous miliary 1mm white nodules were distributed throughout the hepatic parenchyma.

Laboratory Results: A heavy growth of *Listeria monocytogenes* was isolated from the lung, liver, joint fluid, and brain.

Contributor's Morphologic Diagnosis: Acute multifocal purulent necrotizing hepatitis and adrenal adenitis. Acute suppurative bronchopneumonia with intralesional gram positive rods.

Contributor's Comment: In sections of lung, bronchioles are filled with neutrophils, macrophages and cellular debris. Surrounding alveoli are filled with neutrophils, cell debris and fibrin. Occasional keratinized squamous cells also are present in alveoli. Extracellular and intracellular gram-positive rods are present in alveoli and bronchioles. These same bacteria are positive with a *Listeria* immunoperoxidase stain using a goat polyclonal anti-*Listeria* antibody (Kirkegaard & Perry). In sections of liver, there are widely distributed microabscesses composed of aggregates of

degenerate neutrophils and fibrin that replace necrotic hepatocytes. Likewise, there are similar microabscesses throughout the adrenal cortex.

Similar microabscesses were present in the spleen, lymph nodes, myocardium and kidneys. Gram positive rods that were positive with the *Listeria* immunostain were also present in the spleen. The cerebral meninges were diffusely infiltrated with neutrophils.

Listeria monocytogenes is a gram-positive facultative intracellular bacterium. It is a fecal organism, plant saprophyte and animal pathogen. Listeriosis in domestic animals and humans caused by *Listeria* sp. occurs worldwide. Different forms of the disease include abortion, encephalitis, septicemia and rarely iritis, keratoconjunctivitis and mastitis. Infections in cattle have been associated with feeding silage.

There are few reports of neonatal septicemia in foals due to *Listeria monocytogenes*. In one report, the diagnosis was made by blood culture in a 21-day-old Appaloosa filly and the foal recovered after antibiotic therapy. Another case was reported in a one-month-old Arabian foal with combined immunodeficiency. Also, a 6-day-old foal that was presented with diarrhea and later developed pneumonia and septicemia was diagnosed with listeriosis. The exact route of infection is unknown in these cases and in the present case. However, pneumonia was severe in two cases suggesting aspiration or possibly in utero infection. In utero infection is suspected in the present case because the foal was born premature and weak and abortions due to *Listeria* infection are reported in horses. The present case was complicated by uro-abdomen secondary to a ruptured urachus. *Listeria monocytogenes* should be considered in cases of equine neonatal sepsis and in abortions. Culture is needed for a definitive diagnosis. Immunohistochemistry was helpful in substantiating the cause of pneumonia in this case.

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- AFIP Diagnoses:**
1. Lung: Bronchopneumonia, necrosuppurative, subacute, diffuse, severe.
 2. Adrenal gland: Adrenalitis, necrosuppurative, subacute, random, multifocal, moderate.
 3. Liver: Hepatitis, necrosuppurative, subacute, random, multifocal, moderate.

Conference Comment: *Listeria monocytogenes* has a predilection for intestinal tissues, reproductive tissues, and the brainstem. Infection is typically through ingestion, often due to fecal contamination, and has frequently been associated with poorly ensiled feed with a pH greater than 5.5. Once in the intestinal tract, *L. monocytogenes* is internalized by phagocytic cells or M cells; uptake is mediated by internalin, a surface protein. Intracellular survival is aided by listeriolysin-o (similar to streptolysin-o), which lyses phagosomes and ferritin vesicles. *L. monocytogenes*

passes to neighboring cells via plasma membrane protrusions, avoiding the host defense mechanisms. Latency is common and a compromised or immature immune system may be required for widespread dissemination. Septicemia occurs via the bloodstream and results in the embolic distribution characteristic of bacterial infections.

L. monocytogenes can also gain entry through damaged oral, nasal, or ocular mucosa. Organisms enter the neural sheath of peripheral nerve endings and, via the trigeminal nerve, gain access to the central nervous system. The ensuing meningoencephalitis is characterized by focal gliosis, neutrophilic infiltrates (microabscesses), and central necrosis (liquefaction) with a distribution that is restricted to the brainstem, specifically the pons and medulla. The thalamus and cervical spinal cord can be affected as well, but generally less so. The specific distribution of lesions results in a characteristic clinical presentation, which includes unidirectional circling, head pressing, and unilateral facial paralysis (circling disease).

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References: 1. Wallace S, Hathcock T: *Listeria monocytogenes* septicemia in a foal. J Am Vet Med Assoc **207**:1325-1326, 1995
2. Welsh R: Equine abortion caused by *Listeria monocytogenes* serotype 4. J Am Vet Med Assoc. **182**:291, 1983
3. Wilkins P, Marsh P, Acland H, Del Piero F: *Listeria monocytogenes* septicemia in a thoroughbred foal. J Vet Diagn Invest **12**:173-176, 2000

CASE II – 6213 (AFIP 2790111)

Signalment: 27 year-old, female, chimpanzee (*Pan troglodytes*)

History: This chimpanzee presented with loose green diarrhea, inappetence, dehydration and abdominal cramping. Despite treatment, the chimpanzee rapidly deteriorated over a 2-week period and was euthanized. This animal had not been used on any experimental protocol.

Gross Pathology: The animal was mildly dehydrated. Numerous fibrous adhesions were present in the thorax (between caudal aspects of the lung and the diaphragm) and the abdomen (between the small and large intestine and the peritoneal wall). The colon was dilated and filled with green semi-liquid fecal material. The serosa had multiple areas of congestion/hemorrhage; the mucosa had multiple areas of congestion/hemorrhage and/or ulceration.

Laboratory Results: Fecal culture was positive for *Pseudomonas aeruginosa* and negative for *Clostridium difficile* toxin. Fecal exam was positive for *Enterobius* sp and *Balantidium coli*.

RBC:	4.7 x 10 ⁶ /ul	Bands:	2.4 x 10 ³ /ul
WBC:	11.1 x 10 ³ /ul	Metamyelocytes:	0.3 x 10 ³ /ul
Monocytes:	0.2 x 10 ³ /ul		
Lymphocytes:	1.2 x 10 ³ /ul		3 + target cells
Segmented cells:	6.9 x 10 ³ /ul		1 + macrocytes and Döhle bodies
ALT:	130 U/L	CPK:	> 2,000 U/L
AST:	217 U/L	Total protein:	5.8 g/dl
Creatinine:	6.5 mg/dl	Albumin:	1.9 g/dl
BUN:	> 160 mg/dl	Phosphorus:	10.3 mg/dl

Contributor's Morphologic Diagnoses: Colitis, ulcerative, subacute, segmental, severe with intralesional parasites (consistent with *Enterobius* sp and *B. coli*) and bacteria, colon.

Contributor's Comment: Severe inflammation was present in the colon of this chimpanzee. Multiple tissue sections showed a spectrum of changes to include acute and subacute inflammation of the mucosa; mucosal necrosis and/or ulceration; intralesional nematodes, protozoa and bacteria; necrotizing vasculitis with thrombosis in the submucosa; and, peritonitis. In some sections, nematode and protozoal organisms were located in the deep submucosa and outer muscle layers of the gut wall. In occasional sections, nematodes were present in the adjacent mesentery.

Three different types of organisms were identified in lesioned areas to include a nematode pinworm consistent with *Enterobius* sp., the protozoan *Balantidium* and a bacterium (*P. aeruginosa* was cultured from the stool). Because of the number of nematodes present, the unusual location of the nematodes in the deep submucosa and mesentery and the close association of the parasite with the described changes, this worm likely caused the animal's problem. The protozoan and bacteria were considered opportunistic pathogens in the process. In fact, *Pseudomonas* or its toxin may have caused the large areas of mucosal ulceration. In conclusion, while the pinworm is a common parasite of chimpanzees and is usually considered of limited or no importance, there are reports of fatal infections. The same can be said for *B. coli*. Why this otherwise innocuous organism caused problems in this chimpanzee was not determined; however, this animal was not known to be immunosuppressed and had not been on any experimental protocol.

AFIP Diagnoses: Colon: Colitis, ulcerative, chronic, multifocal, transmural, severe, with necrotizing vasculitis, fibrin thrombi, oxyurid nematodes, and ciliates.

Conference Comment: Cardiovascular, respiratory, and gastrointestinal diseases are major causes of morbidity and mortality in nonhuman primates. Many pathogenic organisms such as *Shigella* sp., *Salmonella* sp., *Campylobacter* sp., and *Entamoeba histolytica* are commonly associated with clinical disease of the digestive tract. *Balantidium coli* and *Enterobius* sp. are common inhabitants of the digestive tract and although they have been reported to cause significant disease, are generally considered nonpathogenic commensals. Occasionally associated with immunosuppression or concurrent disease, it is uncertain why their presence appears innocuous in some cases and fatal in others.

Balantidium sp. (phylum Ciliophora) are common in pigs, rodents, and primates. Trophozoites are characterized by surface cilia, a prominent basophilic macronucleus, and contractile vacuoles.

Pinworms (family Oxyuridae) are common in horses (*Oxyuris equi*, *Probstmayria vivipara*) as well as nonhuman primates. *Enterobius anthropopithecii* is most often described in the chimpanzee. *Enterobius* sp. are characterized by lateral alae, platymyarian musculature, and an intestine composed of uninucleate cells. Longitudinal sections demonstrate a rhabditoid esophagus composed of a corpus, isthmus, and bulb.

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References: 1. Gardiner C, Poyton S: An Atlas of Metazoan Parasites in Animal Tissues, pp. 17-18. Armed Forces Institute of Pathology, Washington, DC, 1999
2. Gardiner C, Fayer R, Dubey J: An Atlas of Protozoan Parasites in Animal Tissues, 2nd ed., pp. 16-17. Armed Forces Institute of Pathology, Washington, DC, 1998
3. Holmes D, Kosanke S, White G: Fatal enterobiasis in a chimpanzee. JAVMA **177**:911-912, 1980
4. Hubbard G, Lee D, Eichberg J: Diseases and pathology of chimpanzees at the Southwest Foundation for Biomedical Research. Amer J of Primatol **24**:273-282, 1991
5. Keeling M, McClure H: Pneumococcal meningitis and fatal enterobiasis in a chimpanzee. Lab An Sci **24**:92-95, 1974
6. Schmidt R: Systemic pathology of chimpanzees. J Med Primatol **7**:274-318, 1978
7. Toft J, Eberhard M: Parasitic Diseases. In: Nonhuman Primates in Biomedical Research Diseases, eds. Bennett B, Christian R, Henrickson R, pp. 132-133, 140-141. Academic Press, San Diego, CA, 1998

8. Zhang G, Ji X, McManus D: The presence of pinworms (*Enterobius* sp.) in the mesenteric lymph nodes, liver and lungs of a chimpanzee, *Pan troglodytes*. *J Helminthol* **64**:29-34, 1990

CASE III – DI-01-114 (AFIP 2790168)

Signalment: FVB-wild type, Female, Mouse

History: None

Gross Pathology: Mass within the cervical region.

Laboratory Results: None

Contributor's Morphologic Diagnosis: Salivary glands: Myoepithelioma, malignant.

Contributor's Comment: There are 3 sections of salivary glands (serous acini) with adjacent adipose tissue and striated skeletal muscles. The salivary gland parenchyma is effaced by a non encapsulated, infiltrative mass that extends to the edge of the cut section. The mass is composed of two populations of neoplastic cells (epithelial and mesenchymal origin) that are supported by a fine fibrovascular to myxomatous stroma. The neoplastic epithelial cells are arranged in small islands and packets, cells are round to oval to elongated to irregularly-shaped, 15 to 35 um in diameter, contain a moderate amount of eosinophilic granular to finely vacuolated cytoplasm with poorly defined cytoplasmic boundaries. Nuclei are 10 to 20 um, round to elongate to indented to pleomorphic; some nuclei contain finely granular chromatin while others are more vesicular with 1 to 3 nucleoli. Mitotic figures are frequent (7-14/HPF). The degree of anisocytosis and anisokaryosis is high.

The neoplastic mesenchymal cells are arranged in short bundles, whorls, and sheets. Cells are oval to spindle-shaped, 10 to 30 um in length, contain a moderate amount of eosinophilic finely vacuolated cytoplasm with poorly defined cytoplasmic borders. Nuclei are oval to elongated, 8 to 20 um in length, and contain coarsely clumped chromatin with occasionally 1-2 nucleoli. Mitotic figures are frequent (7-10/HPF). The degree of anisocytosis and anisokaryosis is high. Clusters of neoplastic cells invade lymphatics (metastases). There is mild diffuse infiltration of neutrophils with occasional lymphocytes and plasma cells. There are multifocal to coalescing areas of moderate necrosis that contain necrotic debris, eosinophilic seroproteinaceous material, hypereosinophilic cells with pyknotic nuclei (individual cell necrosis), degenerated and viable neutrophils. There is compression atrophy and fibrosis of the remaining serous acini.

Myoepithelioma arises from myoepithelial cells of various exocrine glands. In mice, these tumors often involve salivary, mammary, clitoral, preputial and harderian glands. When the epithelial component of the tumor cannot be readily identified in the H&E section, immunohistochemistry with vimentin, S-100, smooth muscle actin, and cytokeratin can be used to differentiate the mass from other sarcomas. On the submitted case, immunohistochemistry with smooth muscle actin clearly identified the mesenchymatous component of the tumor. Myoepitheliomas are usually benign but in this case, malignant transformation was suggested by the presence of lymphatic metastases, cellular pleomorphism and high mitotic rate.

AFIP Diagnosis: Salivary gland: Myoepithelioma, malignant.

Conference Comment: Myoepithelial cells are modified epithelial cells that are found in a variety of exocrine glands in which they encircle secretory cells, have contractile properties, and direct secretions into and through duct systems.

Myoepitheliomas are derived from a single cell population but exhibit both spindle and epithelioid features which often blend together imperceptibly. In humans, myoepithelial carcinomas are categorized as epithelioid, clear, hyaline, spindle, or mixed, based upon the predominant cell morphology. Immunohistochemically, myoepithelium can be identified using markers for keratin, smooth muscle actin, and calponin (an actin binding protein).

The differential diagnosis discussed in conference included adenoma and adenocarcinoma which tend to form acini, and tumors induced by the polyoma virus which tend to be multicentric and include both mesenchymal and epithelial components.

Contributor: Pfizer Global Research and Development, Groton CT 06340

References: 1. Fletcher C: Diagnostic histopathology of tumors. vol. 1, pp.168-169. Pearson Professional Limited, 1995
2. Saveria A, Sloman A, Huvos A, Klimstra D: Myoepithelial carcinoma of the salivary glands: a clinicopathologic study of 25 patients. Am J Surg Pathol **24**:761-764, 2000
3. Sundberg J, Hanson C, Roop D, Brown K, Bedigian H: Myoepitheliomas in inbred laboratory mice. Vet Pathol **28**:313-323, 1991

CASE IV – P17A or P17B (AFIP 2790078)

Signalment: 3-week-old C57BL male mouse

History: None

Gross Pathology: Body as a whole: Pronounced bilateral distention of the flanks, emaciation.

Kidneys: Severe bilateral renomegaly (1.2 x 1 x 1 cm). The kidneys are uniformly enlarged, light tan to white and spongy. Innumerable translucent and minute cystic structures are observed throughout the organ.

Laboratory Results: Unavailable

Contributor's Morphologic Diagnosis: Kidney: Polycystic kidney disease.

Contributor's Comment: The kidney is markedly enlarged. There is severe dilation of the vast majority of tubules throughout the cortex and medulla. The corticomedullary junction is obscured. Foci of residual parenchyma are interspersed in low numbers among the cysts, especially in the superficial cortex. Affected tubules are lined by an epithelial monolayer and many are grotesquely distended. The lining epithelium is predominantly attenuated and squamous, but cuboidal epithelium as well as segments where rounded lining cells project into the lumen in a "tombstone" fashion, are also observed. Most cysts are optically empty, but some contain smooth homogenous or flocculent eosinophilic material, at times admixed with scant cellular debris and small amount of granular basophilic material. Cysts abut each other throughout most of the parenchyma, but multifocally, there are areas where variable amounts of expanded and very loose interstitial tissue are encountered. These foci often contain a few residual tubules of relatively normal caliber or only mildly dilated unobtrusive blood vessels, and scant intratubular or extratubular necrotic debris. The number of glomeruli is reduced. Those present exhibit variable, at times severe dilation of Bowman's capsule, while others appear normal. Immature glomeruli are seen in the superficial cortex. Additional findings, not present in all slides, include multifocal subcapsular hemorrhages, multifocal mild capsular fibrosis, mild multifocal perirenal mixed inflammatory cellular infiltration, and severe compression atrophy of the adjacent adrenal gland.

This condition arose spontaneously in one of our C57BL lines. Progressive distention of the flanks was observed and pups either died or were euthanized around weaning at 3 weeks old. One or two male and/or female affected pups per litter with normal parents would suggest an autosomal recessive mode of inheritance. At present we are unable to determine from which segment of the nephron the cysts arose. Studies employing lectin markers, ultrastructural features and embryos may clarify this issue. Extrarenal lesions seen in the mice include bile

duct hyperplasia, and vascular necrosis in multiple organs, the latter presumably due to uremia.

Renal cystic diseases comprise a heterogeneous group of hereditary, developmental, and acquired disorders. In man, polycystic kidney disease (PKD) is a significant cause of morbidity and mortality. Autosomal dominant (ADPKD) and autosomal recessive (ARPKD) forms are recognized. ADPKD is slowly progressive, often associated with a variety of extrarenal manifestations and usually leads to renal failure in late adulthood. ARPKD is rare, often diagnosed in early infancy by massive renomegaly, and is rapidly progressive.

Many spontaneous and experimental (induced either by genetic manipulation or chemicals) murine models of PKD have been described. Study of these models has elucidated some mechanisms of cystogenesis including altered cell proliferation, cell differentiation, extracellular matrix composition and integrin expression. The clinical features and pathological findings in this case closely resemble a condition which arose as a spontaneous mutation in C57BL/6J mice and reported as murine congenital polycystic kidney disease (CPK).

AFIP Diagnosis: Kidney: Cysts, multiple, diffuse (polycystic kidney disease)

Conference Comment: Autosomal dominant polycystic kidney disease, the most common hereditary renal cystic disorder in humans, is caused by distinct mutations in the PKD1, 2, or 3 gene. Independent of which gene is mutated, progressive renal dysfunction often leads to renal failure. Extrarenal manifestations include hepatic, pancreatic, and splenic cysts; cardiac valvular abnormalities; and intracranial saccular aneurisms.

Multiple mouse models have been developed to study both ARPKD (cpk, bpk) and ADPKD (pcy, jck, jcpk, CFWwd). Each model is helpful in understanding the role of growth hormones, cell differentiation, cell proliferation, ionic transport, and oncogene expression in cystogenesis. Regardless of the gene defect, the presenting characteristic morphological features of epithelial cell proliferation, fluid secretion, and altered extracellular matrix are similar. This suggests that multiple genes are involved in normal cell to cell and cell to matrix interactions or that they interfere with a cascade of events resulting in a similar cystic phenotype.

In domestic animals, PKD is most often consistent with ARPKD in that the disease manifests as stillbirths or death within the first few weeks of life due to renal failure. Syndromes resembling both recessive and dominant PKD have been described in domestic dogs and cats. In Persian cats, recent studies report a 45 percent prevalence of PKD. Reported extrarenal manifestations include biliary and pancreatic cysts.

Contributor: Weizmann Institute of Science, Rehobot, Israel

- References:**
1. Beck C, Lavelle R: Feline polycystic kidney disease in Persian and other cats: a prospective study using ultrasonography. *Aust Vet J* **79**:181-184, 2001
 2. Preminger G, Koch W, Fried F, McFarland E, Murphy E, Mandell J: Murine congenital polycystic kidney disease: a model for studying development of cystic disease. *J Urol* **127**:556-560, 1982
 3. Schieren G, PeyR, Bach J, Hafner M, Gretz N: Murine models of polycystic kidney disease. *Nephrol Dial Transplant* **11**(Suppl 6):38-45, 1996
 4. Wu G, D'Agati V, Cai Y, Markowitz G, Park J, Reynolds D, Maeda Y, Le T, Hou H, Kucherlapati R, Edelmann W, Somlo S: Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell* **93**:177-188, 1998

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