The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 20

12 March 2008

Moderator:

Dr. Don Gardner, DVM, DACVP

CASE I – H06/344/110 #2 (AFIP 3041763).

Signalment: 13-year-old, female, Freiberger horse (*Equus caballus*)

History: The horse had colic for 6 days. The horse showed obstipation at the flexura pelvis. Its colon was displaced to the right at the flexura pelvis. The horse showed slight icterus. With ultrasound examination the liver was inconspicuous. The clinicians suspected a primary or secondary hepatopathy.

Gross Pathology: The liver showed severe atrophy of the Lobus hepatis dexter; the whole liver was mildly more friable and was marbled dark and pale brown; on cut section, pus was coming out of the bile ducts; several small (up to 0.5 cm in diameter) white, hard superficial nodules were present (calcifications). In the stomach few *Gasterophilus intestinalis* were present on the pars non-glandularis. The wall of the ileum was mildly thickened (up to 1 cm thick). The left colon ascendens ventralis contained large amounts of dry, dark green, fibrous content (impaction); the mucosa was mildly edematous.

Laboratory Results: The blood values showed elevated liver enzymes.

In the liver, a high content of *Pasteurella* sp. and of a mixed flora were isolated (Institut für Veterinär-Bakteriologie of the University of Bern).

Histopathologic Description:
Liver: Multifocally in the lumen of the bile ducts, in the portal triads and in the surrounding liver parenchyma, there are many degenerate neutrophils and cell debris. The bile ducts are surrounded by moderate to large amounts of connective tissue and many lymphocytes, macrophages, plasma cells and degenerate neutrophils. Multifocally the bile ducts are increased in size and in number (bile duct hyperplasia). They are lined by columnar to cuboidal epithelial cells. Epithelial cells show rarely pyknotic nuclei or karyorrhexis and karyolysis. Many epithelial cells are plump and contain large amount of amphophilic cytoplasm and plump, vesiculated, pale basophilic nuclei. Multifocally there are a lot of bile plugs. There is mild lymphangiectasia and periportal edema. Multifocally there is a mild infiltration of the liver capsule with few lymphocytes and plasma cells.

Contributor's Morphologic Diagnosis:
Liver: Cholangiohepatitis, suppurative, multifocal to coalescing, severe, chronic
Atrophy of the Lobus hepatis dexter
Colon ascendens ventralis, left: Impaction

Contributor's Comme nt: C holangiohepatitis is a sporadic, but common disease of adult horses. Generally, the d isease process is in itiated by an ascen ding b iliary tract infection due to a gram negative rod. In the chronic state ch oleliths can d evelop, cau sing ob struction of t he biliary tree an d lead ing to icterus an d co lic. Cholangiohepatitis may also o ccur secon dary to ch olelithiasis.[4]

The ex act etiology and p athogenesis of cholangiohepatitis in larg e an imals is u nknown. The early stages of the disease are ofte n associated wit h periportal in flammation as well as inflammatio n in t he bile du cts. In sup purative ch olangiohepatitis, t he bacterial infectio n m ay b e distributed th rough portal circulation o r ex tend through the b ile ducts. In non-suppurative c holangiohepatitis, di sease progression i s more likely due to an immune-mediated processes.[7]

Because the hepatocytes a re bei ng de stroyed m ore rapidly than t hey can be replaced, fi brosis be gins t o bridge t he a ffected areas of the liver. As the fibrosis becomes more extensi ve, cholestasis a nd failure of hepatic function may occur. The bile ducts and bile duct epithelium undergo proliferation, which may impair b ile excretion.

On necropsy, the liv er will ap pear firm, pale b rown to green, with prominent irregu lar m arkings on t he cu t surface.

Histopathologically, t wo f orms are de scribed. A suppurative form in which there is ex tensive neutrophilia in the periportal area. T he ne utrophils often contain bacteria. Biliary hyperplasia, loss of hepatocytes, an d fibrosis are also evi dent in the peri portal areas. A non-suppurative form o ccurs in which th e primary cellu lar infiltrate is com posed of mononuclear cel ls, p rimarily lymphocytes, and plasma cells.[2,6]

Early in the disease, clinical signs a re re ferable to the inflammatory p rocesses occurring in t he liver. Th ese inflammatory signs include fever and hepatomegaly, and may lead to co lic and b iliary ob struction. Anorexia follows in cases which present for co lic. Biliary obstruction may th en lead to icteru s and hepatic photosensitization. Hepatic encep halopathy and rel ated signs are rare except in cases of chronic hepatic fibrosis.

Clinical pathologic changes include significantly elevated GGT (600-2500 U/L), slight elevations in AST and SDH relative to t he GGT levels, elevated bile acids , leukocytosis with neutrophilia, h yperfibrinogenemia and hyperproteinemia; coagul ation parameters sh ould be within normal range.[1,5]

AFIP Diagn osis: Liver: Ch olangiohepatitis, chronic-active, diffuse, severe, with bile duct hyperplasia, diffuse bridging fi brosis, a nd c holestasis, F reiberger h orse (*Equus caballus*), equine.

Conference Comment: The contributor gives an excellent ov erview of ch olangiohepatitis in horses. C holangiohepatitis in horses has been reported to occur as a primary disease, or secondarily due to cholelithiasis, duodenal inflammation, intestinal o bstruction, n eoplasia, p arasitism, an d certain to xins su ch as p yrrolizidine alk aloid and those of *Trifolium hybridum* (alsike clover).[3,7,8]

Suppurative c holangiohepatitis i n ho rses i s m ost co m-monly asso ciated with cho lelithiasis, which is thought to result from ascending infections from the small intestine.[2] Cholangiohepatitis an d/or pan creatitis secon dary t o re-flux of du odenal co ntents may occur ac utely as in cases of duodenal obstruction or more chronically, due to either intermittent o utflow obstruction or spasm of t he m ajor duodenal papilla secondary to inflammation.[1] It has also been found that bacteria, bacterial products, and endotox-ins may en ter the liv er through t he po rtal circu lation re-sulting in p eriportal inflammatio n.[2] The bacteria m ost commonly asso ciated with ch olelithiasis an d ch olangio-hepatitis in horses are *Escherichia co li*, *Salmonella* sp, *Aeromonas* sp, and *Citrobacter* sp.[2]

Whether cholelith s occur prior to or following the devel-opment o f cholan giohepatitis h as no t b een determined.[4] The pathogenesis of cho lelith fo rmation is not clear, al-though m ost ch oleliths are rep orted t o contain a mix ed amount of bilirubin, bile pigments, chole sterol este rs, esters of ch olic an d ca rboxylic aci d, cal cium phos phate, and sodium taurodeoxycholate.[3]

In cattle and sheep, cho langiohepatitis has been repo rted to occur due to sporidesmin, a fungal tox in pr oduced by *Pithomyces chartarum*[3], and liver flukes such as *Fasciola hepatica*.

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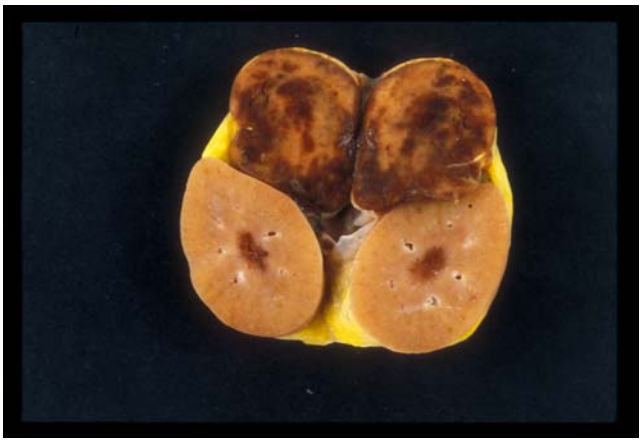
http://www.vetmed.unibe.ch/content/tierpathologie/index_ger.html

References:

1. B uote M: Cholangiohepatitis an d p ancreatitis seco n-dary t o severe g astroduodenal u lceration in a fo al. Can

Vet J 44:746-748, 2003
2. Davis JL, Jones SL: Supp urative ch olangiohepatitis and en teritis in ad ult horses. J Vet Intern Med 17 :583-587, 2003
3. Gerro s TC : Gallb ladder and biliary tract d isease. In: Large Animal Internal Medicine, ed. Smith BP, 2nd ed., pp. 9 46-948. Mo sby-Year Bo ok I nc., New Y ork, NY, 1996
4. Pee k SF, Divers TJ: Medical treatment of ch olangiohepatitis and ch olelithiasis in m ature h orses: 9 cases (1991-1998). Equine Vet J 32:301-306, 2000
5. Ryu SH, Bak UB, Lee CW, Lee YL: Ch olelithiasis associated with recurrent colic in a Thoroughbred mare. J Vet Sci 5:79-82, 2004
6. Schulz KS, Simmons TR, Johnson R: Primary cholangiohepatitis in a horse. Cornell Vet 80:35-40, 1990
7. Smith MR, Steve ns KB, D urham AE, Mar r CM: Equine he patic disease: T he effect of patient- and case-specific va riables on risk and pr ognosis. Eq uine Vet J 35:549-552, 2003
8. Stalker MJ, Hayes MA: Liv er and biliary syste m. In: Jubb, Kennedy, and Palm er's Pat hology of Do mestic Animals, ed. M axie MG, 5t h ed., vol . 2, pp . 345-348. Elsevier Limited, St. Louis, MO, 2007



2-1. Adre nal gl and, cot ton-top t amarin. A n 1. 5 cm di - ameter tan and red mass i n th e typ ical loca tion o f th e right a drenal gl and c ompresses t he a djacent ki dney. Pheochromocytoma. Gross photograph courtesy of New England Re gional Pri mate Researc h C enter, Harv ard Medical School, Southborough, MA http://www.hms.harvard.edu/nerprc/main.html

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CASE II – A03-255 (AFIP 2890562).

Signalment: 17-year-old, m ale, Co tton-top tam arin (*Saguinus oedipus*), nonhuman primate

History: T his elderly tam arin wa s wea k, l ethargic and ataxic. He was dyspneic under ketamine anesthesia. On auscultation there was a cardiac arrhythmia and a systolic ejection murmur that was loudest on the le ft and an EKG demonstrated occasional s kipped beats a nd a bnormal QRS co mplexes. An ultrasound revealed c ystic areas i n the liver and i ncreased m ineralization in the right re nal calyxes. A mass was palpat ed in the right cran ial abdomen. T he an imal was eu thanized du e to a poo r prognosis.

Gross P athology: In the regi on of the ri ght adrenal gland i s a l arge t an a nd red m ass **(Fig. 2- 1)** a pproximately 1.5 cm in diameter. The mass h as a slig htly irregular surface an d is c ompressing the s ubjacent re nal parenchyma. In t he l eft a drenal gl and i s a focal , t an, round approximately 4 mm i n diameter mass. There are numerous cysts in the liver, especially in regions near the diaphragm, fi lled wi th cl ear liquid. T he c ystic regi ons are c oalescing in areas re placing large portions of the hepatic parenchyma. T here are a few tan masses i n the liver measuring 2-4 mm in diameter. Bilaterally th e cortex of t he kidneys i s l ight t an an d co ntains m any sm all cysts. The lungs are edematous and there is pink foam in the trachea. The le ft AV val ve of the heart has mild, irregular thickening of the valve leaflet (endocardiosis).

Laboratory Results : Tumor cells were p ositive fo r chromogranin A, neuron specific enolase (NSE) and synaptophysin.

Tumor cells were n egative for GFAP, VIP, somatostatin, S-100, cytokeratin, NFP and substance P.

Contributor's Morphologic Diagnosis:
Adrenal gland:
1) Pheochromocytoma
2) Myelolipoma (not in all sections)

Contributor's Comment: Pheochromocytomas are neuroendocrine ne oplasms de rived from chr omaffin cel ls of the adrenal medulla and are most common in dogs, cattle and rat s.[2] T he adre nal m edulla deri ves f rom t he neural crest and co nsists o f three t ypes of cells: Ch romaffin, neuronal (ganglion-like), and sustentacular cells.[8] Chromaffin a nd ganglion-like c ells are desce ndent f rom a common sym pathoadrenal neu roblastic p recursor, e xpress neuronal cytoskeletal proteins an d exhibit cat echolaminergic pr operties.[8] Suste ntacular cells are stro-

mal or supportive cells and possess morphologic, functional and antigenic properties similar to those of Schwann and satellite cells.[8]

A diagnosis of a malignant pheochromocytoma in domestic animals is based on invasion of the capsule and adjacent structures (e.g. vena cava) and/or metastasis. In humans, both capsular and vascular invasion may be encountered in benign lesions. Therefore, a diagnosis of malignancy is based exclusively on the presence of metastases.[7] The metastases may involve regional lymph nodes, as well as more distant sites including liver, lung, spleen and bone.[3,7] There are only a few reports of malignant pheochromocytomas with multiple metastases in domestic animals.[10]

Functional pheochromocytomas have been reported infrequently in animals.[2] These tumors may occasionally be associated with clinical signs as a result of the continuous or episodic secretion of one or more of the catecholamines: epinephrine, norepinephrine or dopamine.[2,7] Elevations of blood pressure induced by the sudden release of catecholamines can precipitate acute congestive heart failure, pulmonary edema, myocardial infarction, ventricular fibrillation and cerebral hemorrhage.[2,7] In this case, clinical cardiac abnormalities and histologic findings of myocardial fibrosis (slide not submitted) might suggest catecholamine production by the tumor. Human pheochromocytomas are known to produce sustained hypertension in one-third of cases and experimental catecholamine administration induces myofibrillar degeneration and interstitial fibrosis in animals.[1] However, plasma catecholamine levels and urinary excretion of catecholamines and their metabolites were not measured in this case.

In nonhuman primates adrenal gland tumors are rare. Recognized tumors include: myelolipomas, pheochromocytomas, cortical adenomas, cortical adenocarcinomas, paragangliomas, medullary fibromas and hemangioma/angiomas.[1] In New World nonhuman primates with spontaneous endocrine neoplasia the adrenal gland is most frequently affected with pheochromocytomas reported most often.[5] Other adrenal gland tumors in New World monkeys include myelolipomas.[1] Among prosimian and Old World primates pheochromocytomas have been reported in the ring-tailed lemur (*Lemur catta*), rhesus monkey (*Macaca mulatta*) and cynomolygus monkey (*Macaca fascicularis*).[5] In humans, pheochromocytoma is an uncommon neoplasm and is usually a benign tumor affecting one or both adrenals.[5]

At the New England Primate Research Center, myelolipomas and pheochromocytomas are the two most commonly recognized neoplasms in the adrenal glands of aged Cotton-top tamarins. In humans, most pheochromocytomas occur sporadically in adults with a slight female preponderance.[7] About 10% occur in several, mostly autosomal dominant, familial syndromes including the multiple endocrine neoplasia (MEN) syndromes, type I neurofibromatosis, von Hippel-Lindau disease and Sturge-Weber syndrome.[7] In the familial syndromes, many arise in childhood with a strong male preponderance. Most of the tumors in the syndromes are bilateral (70%), but in the nonfamilial setting only 10-15% are bilateral.[7]

In some of the sections of adrenal from this animal a myelolipoma was also recognized. Myelolipomas are a benign lesion commonly encountered in the adrenal glands of cattle and nonhuman primates and infrequently in other animals.[2] They are composed of accumulations of well-differentiated adipose cells and hematopoietic tissue, including both myeloid and lymphoid elements. Focal areas of mineralization or bone formation may occur. Although the origin of these lesions is uncertain, they appear to develop by metaplastic transformation of cells in the adrenal cortex or cells lining adrenal sinusoids.[2]

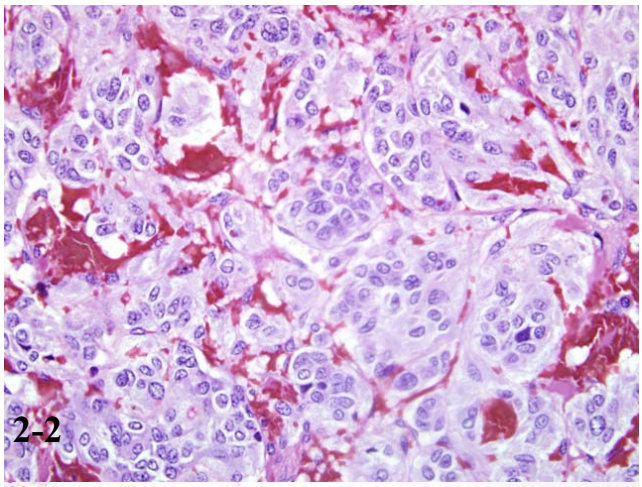
AFIP Diagnosis: Adrenal gland: Pheochromocytoma, Cotton-top tamarin (*Saguinus oedipus*), primate **(Fig. 2-2 and 2-3).**

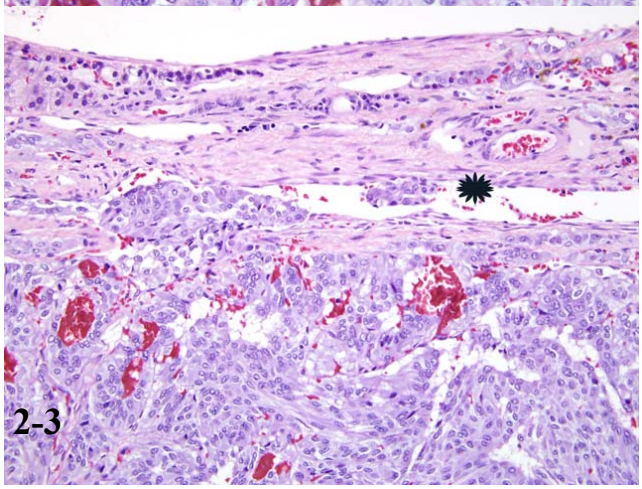
Conference Comment: The contributor gives an excellent overview of pheochromocytomas and their origin. They occur most often in cattle, dogs and some laboratory rats.[2] In dogs, Boxers appear to be overrepresented, and F344 rats with more severe chronic progressive glomerulopathy have been found to have an increased incidence.[2,3] In bulls and humans, pheochromocytomas have been associated with calcitonin secreting C-cell (ultimobranchial) tumors of the thyroid gland.[2]

Tumor development within the adrenal medulla has been associated with multiple factors, including genetics, dietary factors, chronic high levels of growth hormone or prolactin associated with pituitary tumors, and autonomic nervous system stimulation.[3]

In dogs, caval thrombi occur more frequently with pheochromocytomas than adrenal cortical tumors. The caval thrombus primarily develops as an intraluminal extension from the phrenicoabdominal veins rather than by direct invasion of the vena cava.[6]

Rats have three types of chromaffin cells: epinephrine cells, norepinephrine cells, and small granule-containing





cells, unlike human chromaffin cells, which contain both epinephrine and norepinephrine granules within a single cell.[8] Either epinephrine or norepinephrine secreting cells, or both may be found within a pheochromocytoma. Ultrastructurally, norepinephrine granules have an eccentrically placed, small, electron dense core that is surrounded by a wide submembranous space.[2] Epinephrine granules have a coarse granular core that is less dense than that of norepinephrine granules, and has a narrower submembranous space.[2] In dogs, norepinephrine appears to be the principle catecholamine secreted by pheochromocytomas.[2]

Contributor: New England Regional Primate Research Center, Harvard Medical School, Southborough, MA http://www.hms.harvard.edu/nerprc/main.html

References:

1. Brack M: Adrenal gland tumors in two cotton-top tamarins (*Saguinus Oedipus Oedipus*). Lab Anim 34:106-110, 2000

2-2. Adrenal gland, cotton-top tamarin. Neoplastic cells, arranged in nests and packets and supported by a fine fibrovascular stroma, are polygonal with indistinct cell borders, moderate amount of finely granular eosinophilic cytoplasm. There is mild anisokaryosis. (HE 400X).

2-3. Adrenal gland, cotton-top tamarin. There are small variably-sized nests of neoplastic cells within the subcapsular vasculature (star). (HE 400X).

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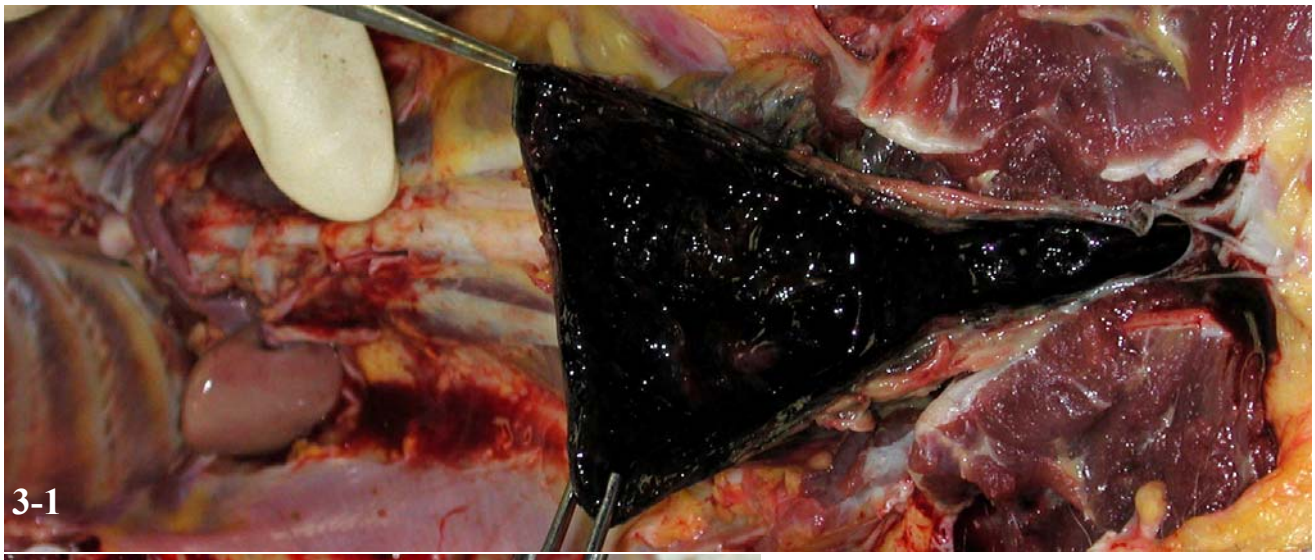
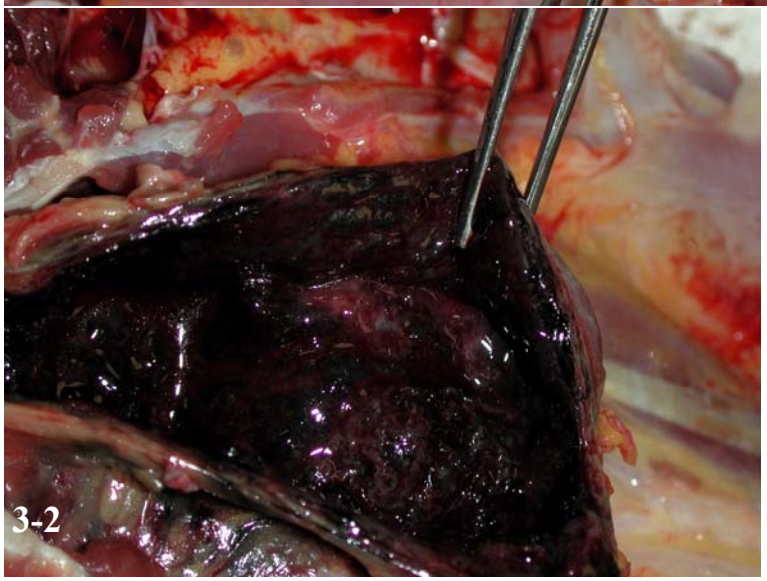
2. Capen CC: Tumors of the endocrine gland. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 629-638. Iowa State Press, Ames, Iowa, 2002
3. Capen CC: Endocrine glands. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 419-422. Elsevier Limited, St. Louis, MO, 2007
4. De Cock HE, MacLachlan NJ: Simultaneous occurrence of multiple neoplasms and hyperplasias in the adrenal and thyroid gland of the horse resembling multiple endocrine neoplasia syndrome: Case report and retrospective identification of additional cases. Vet Pathol 36:633-636, 1999
5. Dias JLC, Montali RJ, Strandberg JD, Johnson LK, Wolff MJ: Endocrine neoplasia in new world primates. J Med Primatol, 25:34-41, 1996
6. Kyles AE, Feldman EC, De Cock HE, Kass PH, Mathews KG, Hardie EM, Nelson RW, Ilkiw JE, Gregory CR: Surgical management of adrenal gland tumors with and without associated tumor thrombi in dogs: 40 cases (1994-2001). J Am Vet Med Assoc 223:654-662, 2003
7. Maitra A, Abbas AK: The endocrine system. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 1219-1223. Elsevier Saunders, Philadelphia, PA, 2005
8. Pace V, Perentes E, Germann P: Pheochromocytomas and ganglioneuromas in the aging rats: Morphological and immunohisochemical characterization. Toxicol Pathol, 30:492-500, 2002
9. Raue F, Frank-Raue K: Multiple endocrine neoplasia type 2: 2007 update. Horm Res, 68:101-104, 2007
10. Sako T, Kitamura N, Kagawa Y, Hirayama K, Morita M, Kurosawa T, Yoshino T, Taniyama H: Immunohistochemical evaluation of a malignant pheochromocytoma in a wolfdog. Vet Pathol, 38:447-450, 2001
11. Vogel P, Fritz D: Cardiomyopathy associated with angiomatous pheochromocytoma in a Rhesus macaque (*Macaca mulatta*). Vet Pathol 40-468-473, 2003





3-1. Urinary bladder, cynomolgus macaque. The urinary bladder contains a mixture of clotted blood and bloody urine.
3-2. Urinary bladder, cynomolgus macaque. The mucosa and muscular wall are necrotic and friable.

Gross photographs courtesy of U.S. Army Medical Research Institute of Infectious Diseases Fort Detrick, MD 21702-5011

CASE III – 040739-16 (AFIP 3073369).

Signalment: 4-year-old, female, cynomolgus macaque (*Macaca fascicularis*).

History: Animal caretakers observed a 4-year-old, female, cynomolgus macaque (*Macaca fascicularis*), colony animal, as being in estrous and not eating well. During physical examination by the laboratory animal veterinarian, the animal was lethargic, dehydrated, and there was a moderate amount of perineal bloody discharge. Initial treatment included oral non-steroidal anti-inflammatory (aspirin) and oral electrolyte replacement. There was no clinical improvement and the regimen was changed to intramuscular flunixin meglumine, intramuscular enrofloxacin, and 300 ml of subcutaneous fluids. The animal's physical condition continued to deteriorate and she became unresponsive and hypothermic. Additionally, increased capillary refill time, bilateral nystagmus, mucoid diarrhea, persistent bleeding from the perineum and significant abdominal pain were observed. The macaque was treated for shock and suspected sepsis using intravenous fluids with 2.75% dextrose and continued intramuscular enrofloxacin and flunixin meglumine. Despite these aggressive therapies, the animal succumbed and a complete necropsy was performed.

Gross Pathology: Gross necropsy findings included moderate bloody discharge from the vulva. The mucus membranes of the lips, mouth, conjunctiva, and sclera were diffusely pale white. The urinary bladder was diffusely green to black, distended, and contained a mixture of dark red to greenish black fluid that was irregularly

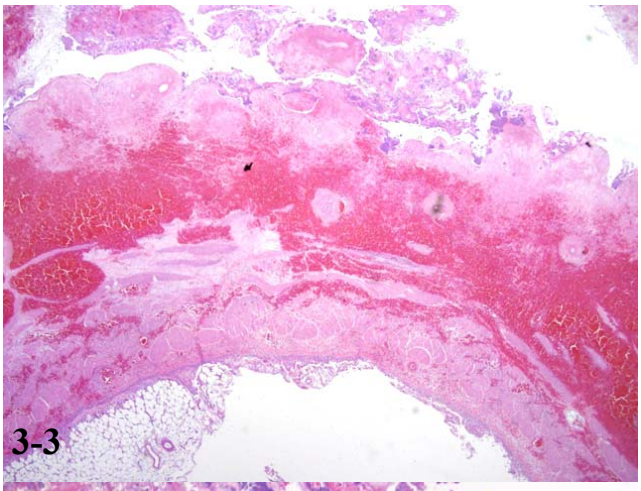
granular to clumped (clotted blood and bloody urine) **(Fig. 3-1).** The mucosa and muscular wall of the urinary bladder were friable and easily torn (necrotic) **(Fig. 3-2).** The hemorrhagic contents from the urinary bladder and the heart blood were sampled at necropsy for microbial culture; the heart blood was extremely thin and watery during collection.

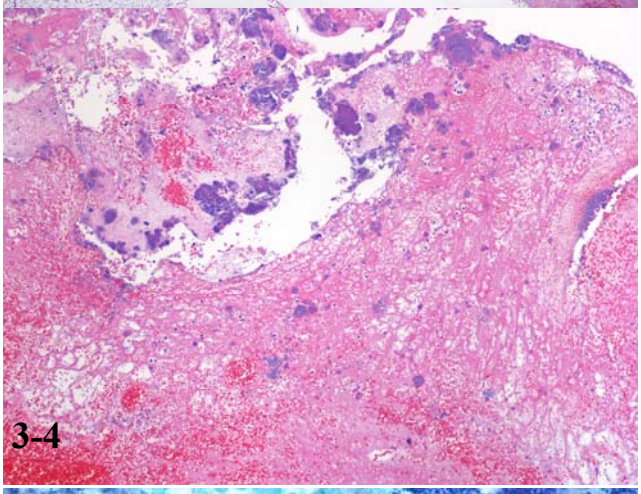
Laboratory Results: Sections were stained by the Gram-Twort staining method. De paraffinized sections were immersed in crystal violet for 1 minute and stained with Lugol's iodine and neutral red/ fast green. Gram-positive organisms stain blue to black and Gram-negative organisms stain pink to red.

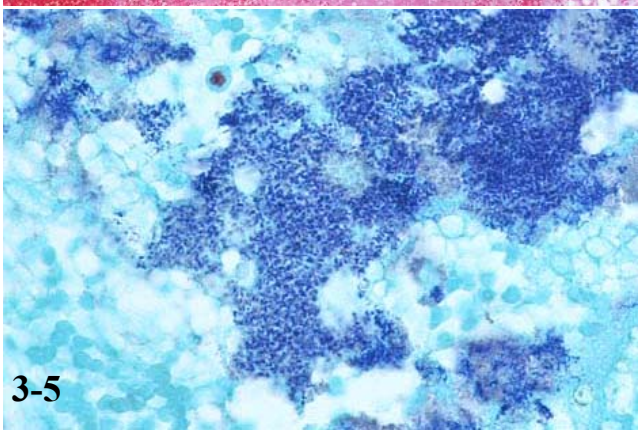
Heart blood culture: No growth after 72 hours
Urine culture: *Corynebacterium* sp.

Histopathologic Description: Within the section of urinary bladder there is transmural necrohemorrhagic to fibrinosuppurative cystitis that is diffuse and severe **(Fig. 3-3).** The urinary bladder mucosa is replaced by a fibrinonecrotic (diphtheritic) membrane or pseudomembrane, composed of abundant fibrin, hemorrhage, necrotic transitional epithelial cells, degenerate neutrophils, and many coryneform bacteria **(Fig. 3-4).** The wall of the urinary bladder (submucosa, muscular tunic, and serosa) is expanded three to four time's normal thickness by abundant fibrin, edema, hemorrhage, and viable and degenerate neutrophils. Blood vessels are congested and there is multifocal necrotizing vasculitis with disruption of architecture in some/all vessel tunics (intima, media, and adventitia) and multifocally blood vessels contain fibrin thrombi.

Additional significant histologic findings in the cynomolgus macaque included acute to subacute fibrinous polyserositis that included the urinary bladder, stomach, pancreas and mesentery. The inflammatory lesions on the serosal surfaces of the viscera were attributed to leakage of the contents from the necrotic urinary bladder into the abdomen (peritonitis).







3-3. Urinary bladder, cynomolgus macaque. Diffusely, there is transmural hemorrhage and loss of urothelium. (HE 20X).
3-4. Urinary bladder, cynomolgus macaque. Multifocally within areas of hemorrhage and necrosis there are large colonies of bacteria. (HE 100X).
3-5. Urinary bladder, cynomolgus macaque. Pleomorphic short rods are Gram-positive. (Gram-Twort method, 400X).

Photomicrographs 3-4 and 3-5 courtesy of U.S. Army Medical Research Institute of Infectious Diseases Fort Detrick, MD 21702-5011

Contributor's Morphologic Diagnoses:
1. Urinary bladder: Cystitis, necrohemorrhagic, transmural, subacute, diffuse, severe, with fibrinonecrotic membrane, hemorrhage, edema, necrotizing vasculitis, thrombi, structural loss (perforation), and many Gram positive coryneform bacteria **(Fig. 3-5).**
2. Urinary bladder, serosa and mesentery: Serositis and peritonitis, necrotizing, subacute, diffuse, severe, with neutrophilic and histiocytic inflammation, marked mesothelial cell hypertrophy and hyperplasia, hemorrhage, fibrin and edema.

Contributor's Comment: Corynebacterial infections are important causes of disease, morbidity, and death in humans and various wild and domestic animal species. *Corynebacterium diphtheriae* causes diphtheria in humans, a highly contagious upper respiratory tract disease characterized by a pseudomembrane within the tonsil, pharynx, and nose. However, many non-diphtheritic coryneform bacteria are a component of the bacterial flora in the skin and mucous membranes, ubiquitous environmental organisms, and potential opportunistic pathogens.[6,7,8] With a few exceptions, the opportunistic infections caused by non-diphtheritic coryneform bacteria are frequently characterized by necrotizing tissue lesions with suppurative inflammation in the affected host.[7,9]

Of the non-diphtheritic coryneform bacteria, several members of the *Corynebacteria renale* group, including *C. renale* (I), *C. pilosum* (II), and *C. cystitidis* (III), are opportunistic urinary tract pathogens in domestic animals and natural causes of cystitis, ureteritis, and ascending pyelonephritis in cattle. This condition in the bovid is commonly known as bacillary pyelonephritis.[6,8,9] Animals may become predisposed to bacillary pyelonephritis through physical or chemical damage to the lower genitourinary tract caused by dystocia, urinary bladder paralysis, and urinary catheterization. Urinary tract infections are more common in females. These predisposing factors disrupt the host's natural defenses, such as the mucosal barrier, and may allow initial colonization of tissue with coryneform bacteria. Hemorrhagic urethritis, cystitis, and pyelonephritis develop as a result of ascending urinary tract infection.[6,8,10,14]

This case displayed similarities to corynebacterial urinary tract infections in other animal species, including natural and experimental infections in cattle, goats, mice and rats.[2,5,6,7,12] Infection with *C. renale* group bacteria in cattle in particular may affect all or part of the urinary tract. In the urinary bladder, typical lesions include mural thickening from infiltrating leukocytes and hemorrhage, vasculitis and fibrin thrombi, mucosal necrosis, ulceration and perforation, hemorrhage; and replacement of the mucosa by a fibrinonecrotic (diphtheritic) membrane.[6,8]

Several bacterial virulence factors may predispose animals to infection and progression of necrohemorrhagic

Table of common pathogenic Corynebacteriae adapted from Jones et al.[6] and Quinn et al.[9]

Organism Principle Species		Disease
Corynebacterium diphtheriae	Human	Diptheria
C. renale (Type I)	Bovine	"Bacillary" pyelonephritis, ureteritis, cystitis, may be recovered from healthy individuals
C. cystitidis (Type II)	Bovine	Hemorrhagic cystitis and pyelonephritis
C. pilosum (Type III)	Bovine	Cystitis and pyelonephritis
C. pseudotuberculosis	Ovine and caprine	Caseous lymphadenitis, produces phospholipase D exotoxin
	Equine	Ulcerative lymphangitis and pectoral abscesses
C. bovis	Bovine	Mastitis (rare), found in teat canal of 20% of apparently healthy dairy cows
C. kutscheri	Rodents	Pseudotuberculosis
C. ulcerans	Non-human primates, and many other species	Bite wounds and abscesses

urinary system lesions due to *C. renale* group organisms. *Corynebacteria renale* group organisms possess pili, allowing attachment to the urogenital mucosa and facilitating ascension of microbes into the bladder and kidneys. The bacteria produce urease and hydrolyze urea, which may be contributory to extensive ulceration of the mucosa and necrosis in the affected tissues.[5,8,9,14] Thus, high protein diets and subsequently elevated urinary urea levels may predispose animals to disease because of the ability to hydrolyze urea.[9]

Host factors may also predispose animals to infection. In cattle, females are more predisposed to infection than males due to anatomic structure, hormonal influences, and risks associated with pregnancy or iatrogenic procedures; infection in bulls is rare.[8] Females have short urethras, which decreases the anatomic barrier bacteria must overcome to reach the urinary bladder and kidneys, and urethral trauma associated with dystocia or catheterization may serve as initiating events for infection.[8,10,14] Hormone-induced changes in female animals may serve as predisposing factors; high estrogen levels may affect the functional integrity of the epithelium in the urethra and urinary bladder; cattle with high estrogen levels that graze pastures are reportedly prone to infection with *C. renale*.[8,9] In some animal species, such as the sow, estrogen causes an elevation in the urine pH, which may produce an alkaline environment optimal for expression of bacterial pili and enhanced microbial survival and proliferation.[8,14] Spontaneous and experimentally induced *C. renale* infections in animals are frequently associated with alkaline urine, although this may be reflective of post infection bacterial hydrolysis of urea and production of ammonia rather than preexisting alkaluria.[5,8,14]

In this case, the histopathologic findings of necrotizing and hemorrhagic cystitis, and the microbial culture results from the contents of the urinary bladder support the underlying cause of death as complications from infection by non-diphtheritic coryneform bacteria. The signalment, clinical history, pathological findings and microbial culture results in this case of necrohemorrhagic cystitis show similarities to spontaneous and experimentally induced corynebacterial urinary tract disease observed in several animal species. The perineal bleeding was presumed to be normal estrous bleeding, therefore, diagnosis and treatment were delayed in this case. Severe urinary tract infection due to corynebacteria should be included in the clinical differential diagnosis for protracted perineal bleeding in macaques.

AFIP Diagnosis: Urinary bladder (per contributor): Cystitis, necrohemorrhagic, transmural, diffuse, severe, with fibrin, edema, and large colonies of bacilli, cynomolgus macaque (*Macaca fascicularis*), primate.

Conference Comment: The contributor gives an extensive overview of *Corynebacterium* infections, their pathogenesis and virulence factors. Although members of the *Corynebacterium renale* group are commonly associated with cystitis and pyelonephritis in cows, they are not generally associated with cases in non-human primates.[13]

In sheep, ulcerative posthitis of wethers (also known as sheath rot or pizzle rot)[9], is a disease that occurs due to the presence of a transmissible urea-hydrolyzing bacterium and the excretion of urine rich in urea.[3] The lesions begin as an ulceration of the prepuce that may progress to destruction of the urethral process and ulceration of the glans penis.[3] *C. renale*, *Rhodococcus equi*, and *C. hofmanni* have all been isolated from infections.[3]

In dogs and cats *Corynebacterium urealyticum*, a urease-producing bacteria, is associated with alkaline urine of pH > 8 and struvite and calcium phosphate precipitations that form encrustations along the bladder wall.[1,11] *Staphylococcus* sp and some strains of *Proteus mirabilis* are more commonly associated with alkaline urine and struvite production in dogs, but do not produce the mucosal encrustations seen with *C. urealyticum*.[1]

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References:

1. Bailiff NL, Westropp JL, Jang SS, Ling GV: *Corynebacterium urealyticum* urinary tract infection indogs and cats: 7 cases (1996-2003). J Am Vet Med Assoc 226:1676-1680, 2005
2. Elias S, Abbas B, El San-Ousi SM: The goat as a model for *Corynebacterium renale* pyelonephritis. Br Vet J 149:485-493, 1993
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, pp. 614-615. Elsevier Limited, St. Louis, MO, 2007
4. Hayashi A, Yanagawa R, Kida H: Adhesion of *Corynebacterium renale* and *Corynebacterium pilosum* to the

epithelial cells of various parts of the bovine urinary tract from the renal pelvis to vulva. Vet Microbiol 10:287-292, 1985
5. Jerusik RJ, Solomon K, Chapman WL, Wooley RE: Experimental rat model for *Corynebacterium renale*-induced pyelonephritis. Infect Immun 18:828-832, 1977
6. Jones TC, Hunt RD, King NW: Diseases caused by bacteria. In: Veterinary Pathology, eds. Thomas CJ, Hunt RD, King NW, pp. 479-481. Lippincott Williams & Wilkins, Boston, MA, 1997
7. Lipsky BA, Goldberger AC, Tompkins LS, Plorde JJ: Infections caused by nondiphtheria corynebacteria. Rev Infect Dis 4:1220-1235, 1982
8. Maxie MG, Newman SJ: Urinary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 490-494. Elsevier Limited, St. Louis, MO, 2007
9. Quinn PJ, Markey BK, Cater ME, Donelly WJC, Leonard FC: *Corynebacterium* species. In: Veterinary Microbiology and Microbial Disease, pp. 55-59. Blackwell Science Ltd., Oxford, 2002
10. Rebhun WC, Dill SG, Perdrizet JA, Hatfield CE: Pyelonephritis in cows: 15 cases (1982-1986). J Am Vet Med Assoc 194:953-955, 1989
11. Saulez MN, Cebra CK, Heidel JR, Walker RD, Singh R, Bird KE: Encrusted cystitis secondary to *Corynebacterium matruchotii* infection in a horse. J Am Vet Med Assoc 226:246-248, 2005
12. Shimono E, Yanagawa R: Experimental model of *Corynebacterium renale* pyelonephritis produced in mice. Infect Immun 16:263-267, 1977
13. Stevens EL, Twenhafel NA, MacLarty AM, Kreiselmeier: Corynebacterial necrohemorrhagic cystitis in two female macaques. J Am Assoc Lab Anim Sci 46:65-69, 2007
14. Yeruham I, Elad D, Avidar Y, Goshen T: A herd level analysis of urinary tract infection in dairy cattle. Vet J 171:172-176, 2006

CASE IV – G7344 (AFIP 3034502).

Signalment: 5-year-old, intact male, rhesus macaque (*Macaca mulatta*), non-human primate.

History: This monkey was inoculated with simian immunodeficiency virus ($SIV_{mac}239$) on 25/08/05 via the tonsillar route. There was a high virus load after two weeks post infection. Twenty-five weeks after the tonsillar challenge the animal showed a pustular skin rash, deteriorating general condition and reduced appetite. The monkey was euthanized on 15/02/06 due to a poor prognosis.

Gross Pathology: At necropsy the rhesus macaque was in a good nutritional condition. The skin was covered with multiple single to coalescing umbilicated pustules. Pustules were preferentially found within the inguinal region, the lips, the hands and feet but they also affected the tongue, the gingiva and the oropharyngeal mucosa. The skin pustules were umbilicated, covered with a central invaginated crust and surrounded by peripheral hyperemia.

Further findings included a severe, necrotising pneumonia and splenitis, a generalized hyperplasia of the lymph nodes and severe follicular hyperplasia of the spleen.

Laboratory Results: Immunohistochemistry: *Herpes simplex* Type 1 and 2: negative

Histopathologic Description: At microscopic examination the skin and the mucous membranes revealed focal areas with epidermal vesiculation, epidermal acanthosis, acantholysis and ballooning degeneration as well as full thickness epidermal necrosis and ulceration. A mixed inflammatory infiltrate composed of neutrophilic and eosinophilic granulocytes, few histiocytes and lymphocytes accompanied the process. In some locations hair follicles and sebaceous glands were involved in the dermal process. Intact affected cells of the vesicle base or margin contained single round to oval intracytoplasmatic inclusion bodies identical with Guarnieri bodies. The Guarnieri bodies were eosinophilic and lay close to the nuclei of infected cells. They were randomly distributed within the altered epithelium. Rare syncytia formations were found close to the basal epithelial layer. At the skin of soles and palms the lesion was arrested in the vesicular stage, covered with a thick intact epidermal cell layer (not included in all sections).

Transmission electron microscopy of skin samples revealed single orthopox like particles in the cytoplasma of keratinocytes.

Monkey pox virus was diagnosed by PCR and cell culture.

Contributor's Morphologic Diagnosis: Tongue: Dermatitis, erosive-ulcerative, subacute, multifocal, severe, with single intracytoplasmatic eosinophilic inclusion bodies, rhesus macaque (*Macaca mulatta*), non-human primate.
Skin: Dermatitis, proliferative, pustular, subacute, multi-

focal, severe, with single eosinophilic intracytoplasmatic basophilic inclusion bodies and rare syncytia, rhesus macaque (*Macaca mulatta*), non-human primate.

Contributor's Comment: Monkeypox is a rare viral disease that is found mostly in the rainforest countries of Central and West Africa. The disease is called "monkeypox" because it was first discovered in laboratory monkeys in 1958.

Monkeypox virus belongs to the orthopox virus group of pox-viruses. Other orthopox viruses that can cause infection in humans and non-human primates include variola (smallpox), vaccinia (used in smallpox vaccine), and cowpox viruses. Pox-viruses are large, complex double stranded DNA viruses.

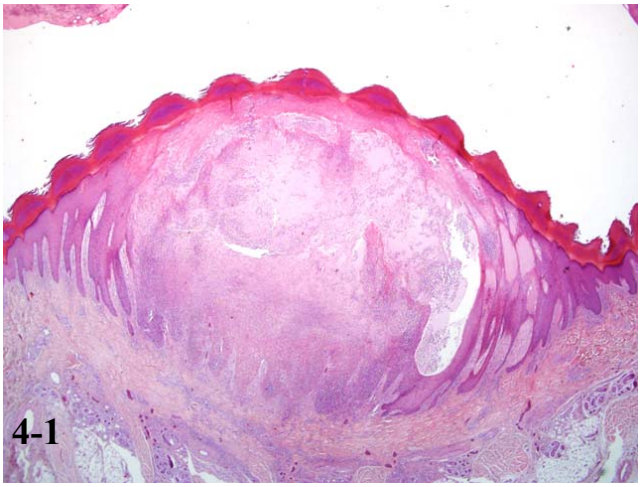
Naturally monkeypox virus only occurs in the tropical rain forest of Western and Central Africa, where it causes subclinical endemic infections in several non-human primate (NHP) species. In the past outbreaks have been reported in captive NHP's, primarily rhesus and cynomolgus, involving institutes importing large numbers of macaques. But the disease has also been reported in marmosets, squirrel monkeys, langurs, baboons, orangutans, gorillas, gibbons and chimpanzees.[6,7,10] The first human case of monkeypox was reported in 1970. Till then several human cases of monkeypox appeared in the tropical rain forest areas of West and Central Africa as isolated cases or as small epidemics. In these regions the infection causes a serious, sometimes fatal smallpox-like disease among young people. Transmission occurs probably aerogenously, by biting or other contacts. People can get monkeypox from an infected animal through a bite or direct contact with the infected animal's blood, body fluids, or lesions (bush meat problem). In 2003 monkeypox was reported among several residents in the United States who became ill after having contact with sick imported prairie dogs. The disease can be spread from person to person too, but it is much less infectious than smallpox.

In non-human primates the disease in usually exhibit a high morbidity and low mortality. Clinical signs may be inapparent or animals may exhibit fever, lymphadenopathy and cutaneous eruptions. Death is uncommon except in infant monkeys. Typical pocks appear as papules of 1 to 4 mm in diameter, which then develop into pustules containing cell debris. The pustules become umbilicated and covered by crusts. The most common sites of pock formation in the monkeys are the face, hands and feet, the mucous membranes of the oral cavity and the genital tract, but also pharynx, larynx trachea, lung, spleen and lymph nodes are commonly involved.

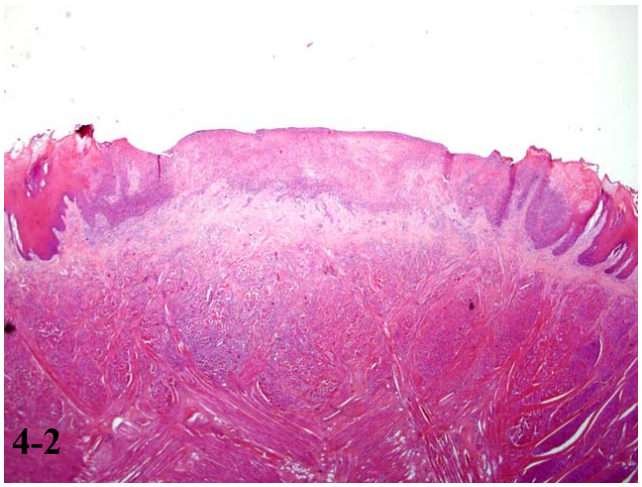
Today we gained experience with the outcome of the disease in immunocompromised monkeys. This accidental case of monkeypox in an immunocompromised animal described here showed that the disease outcome was characterized by severe vesicular exanthema. The skin rash was accompanied by severe respiratory tract involvement and progression of the disease was fatal. Till now it is not clear how transmission occurred in this case. Diagnosis was complicated due to the minimal content of inclusion bodies indicative for poxvirus infection. By electron microscopy typical orthopox like viral particles were demonstrable. An *Eczema herpeticatum* was considered as differential diagnosis, but immunohistochemistry for *Herpes simplex* type 1 and 2 was negative.

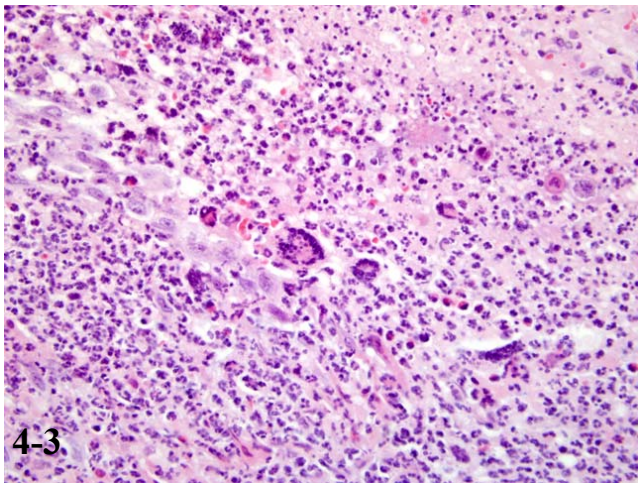
AFIP Diagnosis: 1. Glabrous skin: Dermatitis, vesiculopustular, focally extensive, marked, with acanthosis and ballooning degeneration, rhesus macaque (*Macaca mulatta*), primate **(Fig. 4-1).**
2. Haired skin: Dermatitis, necroulcerative, neutrophilic and eosinophilic, focally extensive, severe with ballooning degeneration.
3. Tongue: Glossitis, necroulcerative, neutrophilic and eosinophilic, multifocal, marked, with ballooning degeneration and intralesional cocci **(Fig. 4-2 and 4-3)**.

Conference Comment: In 2003, several people in the Midwestern United States were diagnosed with monkeypox virus infection. All affected individuals were associated with exposure to captive prairie dogs that had been housed with Gambian giant pouched rats (*Cricetomys* sp.), rope squirrels (*Funiscuirus* spp.), and/or dormice



4-1. Foot, rhesus macaque (Macaca mulatta). Within the epidermis there is a focally extensive vesiculopustule. (HE 20X).





4-2. Tongue, rhesus macaque (Macaca mulatta). Within the mucosal epithelium there is a focally extensive ulcer. (HE 20X).
4-3. Tongue, rhesus macaque (Macaca mulatta). Within necrotic areas there are high numbers of nondegenerate neutrophils admixed with fewer histiocytes, lymphocyte, eosinophils and rare multinucleated syncytial cells. (HE 400X).

(*Graphiurus* sp.) that originated from Ghana.[2,4] As of 30 July 2003, 72 human cases had been reported of human monkeypox virus infection.[2] Affected individuals included veterinarians, pet store personnel, an animal distributor, and children and parents that bought the infected rodents.[2]

Ultrastructurally, orthopox viruses are 375 X 200 nm particles, located free in the cytoplasm, composed of an outer membrane enclosing a characteristic dumbbell-shaped inner electron lucent core that is bounded by two lateral bodies.[4]

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References:

1. Arita T, Jezek Z, Khodakevich L, Ruti K: Human monkey pox: A newly emerged orthopoxvirus zoonosis in the tropical rain forests of Africa. Am J Trop Med Hyg 34:781-789, 1985
2. Bernard SM, Anderson SA: Qualitative assessment of risk for monkeypox associated with domestic trade in certain animal species, United States. Emerg Infect Dis 12:1827-1833, 2006
3. Ginn PE, Mansell JE, Rakich PM: Skin and appendages. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 664-674. Elsevier Limited, St. Louis, MO, 2007
4. Langohr IM, Stevenson GW, Thacker HL, Regnery RL: Extensive lesions of monkeypox in a prairie dog (*Cynomys* sp.). Vet Pathol 41-702-707, 2004
5. Mätz-Rensing K, Ellerbrok H, Ehlers B, Pauli G, Floto A, Alex M, Czerny CP, Kaup FJ: Fatal poxvirus outbreak in a colony of new world monkeys. Vet Pathol 43:212-218, 2006
6. McConnell SJ, Hickman RL, Wooding WL, Huxsoll DL: Monkey pox: Experimental infection in chimpanzees and immunization with vaccinia virus. Am J Vet Res 29:1675-1680, 1968
7. Marennikova SS, Shelukhina EM, Maltseva VI, Ladnnyj ID: Poxviruses isolated from clinically ill and asymptomatically infected monkeys and a chimpanzee. Bull WHO 46:613-620, 1976
8. Percy DH, Barthold SW: Mouse. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 25-28. Blackwell Publishing, Ames, IA, 2007
9. Peters JC: An epizootic of monkey pox at Rotterdam Zoo. Int Zool Yearb 6:274-275, 1966
10. Von Magnus P, Andersen EK, Petersen KB, Birch-Anderson A: A pox like disease in cynomolgus monkeys. Acta Pathol Microbiol Scand 46:156-176, 1959

Orthopoxvirus diseases of animals. Table extracted from Ginn et al.[3]

Orthopoxvirus	**Key points**
Camelpox virus	Dromedary camels; clinically identical to camel contagious ecthyma (parapox)
Cowpox virus	Cutaneous and occasionally respiratory lesions in domestic cats; on face and forepaws; affects wild and domestic Felidae, cattle, dogs, rodents, humans; not endemic in cattle, and infections in cattle are uncommon; wild rodents are the reservoir; severe fatal pneumonia in elephants
Ectromelia virus (mousepox virus)[8]	Limb amputation in surviving mice; systemic infection
Monkeypox virus	Rodents, New Wold monkeys, and great apes; systemic disease
Buffalopox virus	Affects waterbuffalo in India; Zebu cattle apparently refractory to infection; closely related to Vaccinia virus
Uasin Gishu disease virus (unassigned)	Horsepox became naturally extinct in 19th century; recent uncharacterized orthopox viruses isolated from horses with equine papular dermatitis, and in equines with Uasin Gishu disease in Kenya; are found closely related to Vaccinia virus and cowpox virus
Vaccinia virus	Does not cause natural infection in domestic animals
Variola virus (human smallpox)	Affects humans and non-human primates; irradicated?