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Department of Veterinary Pathology
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CASE I – 210726-39/39A/39C (AFIP 2789813)

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

History: This macaque was one of a group of monkeys which were intravenously inoculated with a monkeypox virus cell lysate suspension. All monkeys were observed and given thorough physical examinations on a daily basis. Scheduled blood draws for hematology and serum chemistry assays were also done at the time of exam. This monkey died 14 days post infection.

Gross Pathology: A generalized, multifocally coalescing, exanthematous rash covered most of the skin, and also affected the tongue, gingiva, buccal and oropharyngeal mucosa. At the time of death, these lesions varied from vesicles to pustules, many of which had ulcerated and/or become umbilicated. Notable facial edema was evident. Eyelids, nares, and rectal mucosa were also involved; and a small amount of hemorrhage originated from the rectum. Peripheral lymphadenopathy was marked; and involved submandibular, axillary, inguinal, and popliteal lymph nodes. Small hemorrhagic foci were scattered throughout the lungs. Multiple plaque-like lesions (necrotizing thymitis) were evident along the pericardial surface; and crateriform lesions were occasionally seen in the esophageal mucosa.

Laboratory Results: Not provided.

Contributor's Morphologic Diagnosis: Esophagus: Esophagitis, necroulcerative, multifocal, severe, with epithelial intracellular edema (ballooning degeneration), epithelial syncytia, colonies of bacteria, and basophilic intracytoplasmic inclusion bodies, cynomolgus macaque (*Macaca fascicularis*).

Contributor's Comment: The monkeypox virus (genus *Orthopoxvirus)* and the zoonotic disease it causes was first recognized animals during two outbreaks in wild-caught cynomolgus macaques in 1958 by von Magnus. Other outbreaks of monkeypox in nonhuman primates were retrospectively identified as occurring between 1958-1968, and involved Asian, African, and New World species. However, Orthopoxvirus antibodies were only found in African species of monkeys and squirrels. It is believed that monkeypox virus is only found naturally in Africa; it maintains an enzootic cycle in the forests of central and western Africa.

The first human case of monkeypox was documented in Zaire by Ladnyj in 1970. Only a few hundred cases were reported during the period 1981-1986; however, during 1996-1997 monkeypox reemerged in impressive fashion resulting in several hundred human monkeypox cases. In humans, the disease syndrome (i.e. rash, fever, respiratory signs, and occasional death) is similar to smallpox. Epidemiologically, the significant finding distinguishing this outbreak from that ten years earlier, was that secondary transmission, that is, person-to-person, apparently accounted for the majority of cases. Another significant point to remember is that the global smallpox eradication vaccination program ended shortly after 1979, therefore, there is an ever-increasing population vulnerable to orthopoxvirus infection. Additionally, there are no licensed anti-poxvirus therapeutics, world-wide stocks of remaining vaccine are low, and more importantly, the reinstitution of a vaccination program in Africa using standard smallpox vaccine (vaccinia) would not be without significant risk due to the rising numbers of HIV-infected people.

Considering that monkeypox in humans is a reemerging disease, development of licensed anti-poxviral therapeutics, and new generation vaccine candidates have become a priority. With that said, pertinent animal models must be developed for safety and efficacy studies. Two nonhuman primate models of monkeypox are being developed: an aerosol exposure model, and the intravenous exposure model, as in this case.

Experimental monkeypox viremia causes disseminated disease and generalized rash (exanthem). During viremia the virus can be either associated with monocytes (cell-associated) or free in the plasma, and is able to cross small vessels and capillary walls. In this fashion, the infection spreads from the vessel to adjacent lamina propria and overlying stratum germinativum. A similar process occurs in the skin. The esophageal lesion (enanthema) shown here is fairly typical due to the absence of cornified epithelium which precludes the development of a vesicle or pustule. Infection of epithelial cells results in viral replication, formation of variably sized basophilic intracytoplasmic inclusions (Guarnieri bodies a.k.a. B-type inclusions), development of cell-associated enveloped virus and cell-to-cell extension of the infection, release of extracellular enveloped virus, cellular degeneration, intra- (ballooning degeneration) and intercellular edema, lysis of

membranous cellular structures, and cytolysis and release of immature and mature virions. Since endothelial injury is probable, thrombosis and infarction may contribute to certain lesions. The uninfected esophageal mucosal epithelium is thickened most likely due to the monkey's anorexia, and there are mitotic figures throughout the stratum basale. Immunolabeled viral antigen is evident in a range of cell types, including epithelial, endothelial, monocytic, histiocytic, and other mesenchymal cells such as smooth muscle and fibrocytic.

AFIP Diagnosis: Esophagus: Esophagitis, proliferative and ulcerative, subacute to chronic, multifocal, moderate, with ballooning degeneration, intracytoplasmic eosinophilic inclusion bodies and rare syncytia, cynomolgus macaque (*Macaca fascicularis*), nonhuman primate.

Conference Comment: Poxviruses are large, complex, double stranded DNA viruses. The family Poxviridae is divided into the subfamilies Entomopoxviridae, which infect insects, and Chorodopoxviridae which infect vertebrates. In veterinary medicine, the significant genera of Chorodopoxviridae include orthopoxvirus, parapoxvirus, avipoxvirus, capripoxvirus, suipoxvirus, and leporipoxvirus. The orthopoxviruses are epitheliotropic, antigenically related, and provide cross-protective immunity. Lesions are characterized by vesicle formation and intracytoplasmic inclusion bodies.

Conference participants commented on the presence of abundant mixed bacteria; a common sequela to poxviral ulceration. Intraepithelial nematodes were present in some sections. The histomorphology and location of the nematode is consistent with *Gongylonema* sp.

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CASE II – 200721-2/2A/2B/2D (AFIP 2789825)

Signalment: Female, Strain-13 guinea pig (*Cavia porcellus*), rodent.

History: This guinea pig originated from a closed colony located at our facility. The guinea pig was lethargic and had labored breathing. The animal was euthanatized with euthasol and necropsied.

Gross Pathology: Multifocal areas of pulmonary consolidation primarily confined to the cranial lobes and the hilar region. The remainder of the tissues were within normal limits.

Laboratory Results: None

Contributor's Morphologic Diagnosis: Lung: Bronchopneumonia, necrotizing, multifocal, moderate, with intranuclear inclusions.

Contributor's Comment: Multifocally, bronchi and bronchioles are partially or completely occluded by moderate to abundant numbers of viable and degenerative neutrophils, sloughed epithelial cells, and necrotic cellular debris. Airway epithelium is variably eroded or hyperplastic depending on the section examined. Predominantly within sloughed and to a lesser extent within intact epithelial cells are 3-10 um homogenous, basophilic intranuclear inclusion bodies. Lymphocytes, macrophages, and lesser numbers of plasma cells expand the perivascular and peribronchiolar interstitium and variably extend outward into the adjacent parenchyma. Alveolar septal walls are mildly thickened by proliferating lining cells and/or expanded by red blood cells, lymphocytes, and macrophages. Alveoli are often filled with edema, fibrin, and previously described inflammatory cells. The mesothelium is multifocally hypertrophied. Immunohistochemical staining demonstrates intact and degenerated adenovirus-positive airway epithelial cells. Transmission electron microscopy revealed 70-90 nm viral particles arranged in paracrystalline arrays consistent with an adenovirus. There were no microscopic lesions in the remainder of the tissues examined.

Adenoviruses are nonenveloped, icosahedral dsDNA viruses that affect a variety of species. Mammalian and avian adenoviruses are classified into two distinct genera, designated *Mastadenovirus* and *Aviadenovirus*, respectively. Guinea pig adenovirus (GPAdV) was first reported in 1981 in Germany and was characterized as a disease of low morbidity and high mortality. It is considered a subclinical disease that requires some precipitating event for the disease to develop its full course and cause death. The precipitating factor in this single case of GPAdV presented here is unknown. This guinea pig was not on study and

otherwise appeared in good condition. Typically, the infection in guinea pigs centers on large and small airways with partial or complete occlusion of airway lumina by necrotic debris, sloughed epithelial cells, and inflammatory cells. Large basophilic intranuclear inclusions are present primarily within sloughed epithelial cells and to a lesser extent in the intact epithelium. Ultrastructurally, the virus arranges in paracrystalline arrays within the nucleus; intranuclear crystal inclusions may also be present.

AFIP Diagnosis: Lung: Pneumonia, bronchointerstitial, subacute, multifocal, moderate, with bronchiolar epithelial necrosis and large basophilic intranuclear inclusion bodies, etiology consistent with adenovirus, guinea pig (*Cavia porcellus*), rodent.

Conference Comment: The most frequent single cause of death in laboratory guinea pigs is pneumonia and the most common cause is bacterial infection. Less commonly, as in this case, there are sporadic outbreaks of viral pneumonia. The sporadic nature of adenoviral infections along with the inability to cultivate the organism in vitro has hampered characterization of the virus.

The differential diagnosis discussed during conference included viral and bacterial pneumonias. Cytomegalovirus, a beta-herpesvirus, can result in an acute interstitial pneumonia and produces both intranuclear and intracytoplasmic inclusion bodies. *Bordetella bronchiseptica* (gram-negative bacillus) affects guinea pigs of all ages, causes severe disease and mortality, and results in a suppurative bronchopneumonia with mucopurulent exudates. *Streptococcus zooepidemicus* (gram-positive coccus) is a normal inhabitant of the upper respiratory tract. Bite wounds, oral abrasions, and occasionally inhalation allows drainage of the organism to the local lymph nodes. Although any lymph node can become infected, the cervical lymph nodes commonly are, hence the term cervical lymphadenitis. Septicemia and a suppurative pneumonia can occur. Other sites commonly seeded include the inner ear, joints, serosal surfaces, and kidneys. *Streptococcus pneumoniae* (gram-positive coccus) is arranged in pairs and chains, spreads through aerosol transmission, and can result in a fibrinopurulent pleuropneumonia with pulmonary consolidation and thrombosis of pulmonary vessels. Abortion, arthritis, and osteomyelitis can occur. Additional bacterial pneumonias reported include *Staphylococcus* sp., *Klebsiella pneumoniae*, and *Pasturella* sp.

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CASE III – B1674 (AFIP 2788006)

Signalment: 19-year-old, Flekveih cow, (*Bos taurus*)

History: The cow initially presented with multiple confluent papillary exophytic cutaneous growths on the skin of the mammary gland and teats of more than a years duration. Small biopsy fragments submitted at that time were diagnosed as cutaneous papilloma with focal areas of dysplasia. Multiple cryotherapy treatments with liquid nitrogen resulted in substantial reduction is size and number of skin lesions, but the papillomatous skin masses never completely resolved. The cow was presented again nearly a year later with similar appearing more extensive coalescent papillomatous skin masses, many of which were necrotic and seeped serum. The intervening nonpigmented skin was irregularly roughened with variable sized 3-10 mm raised plaque like lesions.

Gross Pathology: The mammary gland was removed surgically. Numerous coalescent nodular papillomatous growths measuring up to 8cm were scattered over the skin of the mammary gland and teats. The non-pigmented skin between the masses was rough and scaly with raise white plaque like lesions 2-6mm in diameter. The supramammary lymph nodes were enlarged and firm. On cut sections firm whitish-yellow masses were evident irregularly extending from the cortex into the medulla.

Laboratory Results: None

Contributor's Morphologic Diagnosis: Squamous cell carcinoma

Contributor's Comment: Squamous cell carcinomas are the second most common cause of neoplasia in cattle behind lymphosarcoma. In beef breeds with non-pigmented periocular skin squamous cell carcinomas can be common, and are attributed to sun damage. Similar UV light induced lesions have been reported in the poorly pigmented vulvas of cattle and sheep as well as ears of sheep. Solar induced skin damage is also seen on the udder skin of lightly pigmented breeds, especially in higher altitudes of the mountainous regions of the western US and Canada that experience significant snowfall resulting in reflective sunlight damage to the thinly haired mammary skin. This cow was originally purchased from British Columbia, Canada. Squamous cell carcinomas in cattle have reportedly arisen from pre-existing viral induced papillomas, as could have conceivably happened in this case.

AFIP Diagnoses: 1. Haired skin: Squamous cell carcinoma, Flekveih cow (*Bos taurus*), bovine.
2. Nonhaired skin (teat): Mamillitis, perivascular, lymphocytic, diffuse, mild, with multifocal epidermal hydropic degeneration.

Conference Comment: Bovine papillomaviruses are nonenveloped double stranded DNA viruses of the family Papovaviridae. Papilloma formation is the result of viral transcription of mRNAs in the host nucleus. Viral enzymes and proteins result in basal cell hyperplasia, delayed maturation of the stratum spinosum and granulosum, and cytopathic effects. Spontaneous regression is mediated by CD4+ T cells; IgG immunoglobulin is protective against reinfection.

Incorporation of viral DNA into the host genome can result in malignant transformation. Disruption of the viral transcription genes E1/E2, which have an inhibitory effect on E6 and E7 genes, result in overproduction of the E6 and E7 proteins. E7 protein binds retinoblastoma protein displacing E2F transcription factor initiating entry into the S phase of the cell cycle. E6 protein binds p53 targeting it for the ubiquitin proteolysis pathway.

In addition to solar irradiation, topical carcinogens, and viruses, a recent study reported the occurrence of skin tumors following freeze branding and heat branding. Squamous cell carcinomas (SCC) developed at the branding site in seven cattle, six to eight years following branding. In five sheep, SCC developed four to six years after ear branding. In humans, both SCC and soft tissue sarcomas are reported following chronically ulcerated skin and burn scars. These scars that undergo malignant transformation, generally 20 to 60 years after the incident, are referred to as Marjolin's ulcers.

Contributor: Mississippi State University, College of Veterinary Medicine, MS 39762-9825

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CASE IV – USFM-2 (AFIP 2790165)

Signalment: 18-month-old, castrated male, mixed breed, bovine

History: These tissues are from a 112 kg calf which was part of an experiment for the reproduction of the intoxication by the poisonous plant *Indigofera suffruticosa* (Leg. Papilionoideae). The calf was force-fed daily doses of 40g/kg/bw of the aereal parts of the plant for five days. Twenty-two hours after the first feeding, the calf developed hemoglobinuria that persisted until the 5th day of the feeding trial when the calf was euthanatized. Other clinical signs included apathy, pale mucous membranes, rough hair coat, anorexia, decreased frequency and intensity of rumen movements, marked tachycardia (140 bpm) with disturbances in cardiac rhythm and intensity and disturbances in the frequency and intensity of respiratory movements. Before the hemolytic crisis the urine was blue or green and became red-wine after the hemolysis ensued. The prepucial mucosa was bluish due to excretion of a blue pigment present in the plant.

Gross Pathology: The cadaver was anemic. The mucous membranes were pale and the blood was thin and watery. The bladder contained red-wine urine. The kidneys were swollen and dark-brown and the liver has marked lobular pattern and a blue hue in the capsular and cut surfaces.

Laboratory Results: The following hematological data were obtained

		Day of experiment				
	Reference values	0*	2	3	4	5
PCV (%)	24.0-40.0	30	nd	24	16	13
RBCs (10^6/mm^3)	5.0-10.0	6.0	nd	4.3	2.8	2.0
Hemoglobin (g/dl)	8.0-15.0	9.0	nd	6.0	4.6	3.2

MCV (fl)	50.0-60.0	50.0	nd	55.8	57.1	65.0
MCHC (%)	30.0-36.0	30.0	nd	25.0	28.7	24.6
Hemoglobinuria**	-	-	+ +	+ + +	+ + +	+ + +

*Day zero is the day before the administration of the plant
PCV = packed cell volume, RBCs = red blood cells, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration.
**- absent, + mild, + + moderate, + + + marked
nd = not determined

Contributor's Morphologic Diagnosis: 1. Kidney, degeneration of the tubular epithelium moderate, diffuse with proteinosis (hemoglobin) and hyalin droplets in the cytoplasm of the tubular epithelium (hemoglobinuric nephrosis).
2. Liver, centrolobular coagulative necrosis and midzonal vacuolar degeneration, acute moderate. Etiology: Poisoning by *Indigofera suffruticosa*.

Contributor's Comment: A disease in cattle of any age and both sexes, characterized by hemolytic anemia, hemoglobinuria, and abortions has been recognized by cattle owners in various parts of Northeastern Brazil for decades. Cattle owners from those regions reported that the disease occurred in cattle grazing pastures where a plant known by the common names "anil" or "anileira" (*Indigofera suffruticosa*) grows. The disease is seen particularly in those years when the plant proliferates abundantly invading native pastures. In Portuguese "anil" means a blue pigment (indigo, anile) and "anileira" means a tree that produces such blue pigment. The ingestion of *I. suffruticosa* was recently experimentally determined as the cause of the disease by feeding of the aereal parts of the plant to calves. In the spontaneous disease morbidity is around 50% but mortality rates are very low since cattle are withdrawn from the infested pastures after the first clinical signs; recovery ensues. However, economical losses due to weight loss, decrease in milk yield, and costs with treatment are considerable. Only severely anemic cattle will eventually die.

As one can see by the hematological data in this case, the poisoning by *I. suffruticosa* in cattle is characterized by an acute hypochromic macrocytic anemia with signs of intense marrow regeneration. The clinical signs described for the intoxication are compatible with an intravascular hemolytic process in that there is hemoglobinemia and resultant hemoglobinuria. Since few animals die, necropsy data is scant.

The main microscopic findings in the calf from this report were observed in the kidneys and in the liver. In the kidneys there is degeneration and necrosis of epithelial tubular cells associated with great amounts of proteinaceous filtrate (hemoglobin) in the urinary space and tubular lumina. Hyalin droplets are observed in great numbers within the cytoplasm of epithelial cells. Hyalin and granular casts

are also observed in great numbers in the tubules and ducts of the medulla. In the liver there are centrolobular or paracentral areas of coagulative necrosis characterized by marked acidophilic cytoplasm and karyopyknosis and karyorrhexis. These necrotic areas are surrounded by degenerated hepatocytes (swollen and microvacuolated cytoplasm). Mild bile stasis can also be observed. The necrotic and degenerative hepatic lesions are attributed to the anoxia due to the anemia.

Indigofera suffruticosa induces in cattle a toxic hemolytic anemia, the pathogenesis of which is not yet completely determined, but is most probably similar to that of other toxic hemolytic anemias described in domestic animals. In Brazil, other causes of toxic hemolytic anemia reported in ruminants include the ingestion of the plants *Brachiaria radicans* and *Ditaxis desertorum* in cattle and chronic copper poisoning in sheep and cattle. The clinicopathological features of *Brachiaria radicans* and *Ditaxis desertorum* are very similar to those reported in *I. suffruticosa* poisoning in cattle. In other countries the ingestion of wilted or dried leaves of red maple (*Acer rubrum*) has been associated with toxic causes of hemolytic anemia in horses. Other toxic causes of hemolytic anemia in various species of domestic animals include onions (regular and wild varieties), *Brassica* sp. (cabbage, turnip, mustards, kale), and chemicals such as propylene glycol, methylene blue, and phenothiazine. All these plants and chemicals are capable of inducing hemolytic disturbances through the disruption of the red blood cell antioxidative metabolism that leads to the production of methemoglobin and, later, to the development of an erythrocyte inclusion known as Heinz body. Heinz bodies appear similar to a small bubble and are formed through the denaturation of methemoglobin. Several oxidative substances contained in poisonous plants have the ability of inducing these changes.

Glutathione is responsible for maintaining hemoglobin in its reduced form, i.e., by not allowing the transformation from hemoglobin to methemoglobin. That means that any decrease in the levels of glutathione may trigger Heinz body formation. Most of the time, the hemolytic disturbance is caused by a disruption in the normal sequence of the energy metabolism, i.e., by inhibition of one of the enzymes involved in anaerobic glycolysis (Embden Meyerhof pathway) or in the pentose phosphate pathway (derivation from hexose monophosphate). This enzymatic inhibition leads to decreased ATP, NADH, and NADPH synthesis; these substances are paramount in the reduction of oxidized glutathione. The fall in the levels of reduced glutathione impairs the antioxidative metabolism and, as a consequence, induces the transformation of hemoglobin into methemoglobin; the denaturation of methemoglobin results in Heinz bodies formation. It is proposed that this is the pathogenetic mechanism through which all the plants and chemicals mentioned above induce Heinz bodies methemoglobinemia and subsequent hemolysis. The hematological characteristics of Heinz body hemolytic anemias include regenerative anemia, polychromasia, anisocytosis, and metarubricytemia. Another hallmark of this kind of anemia is the brown discoloration of the blood.

Methemoglobinemia and methemoglobinuria can be observed. Heinz bodies are best visualized in blood films stained by supravital methods, although in severe cases they can be spotted in routine stains. Another important hematological feature is the finding of eccentrocytes which are erythrocytes that have lost their Heinz bodies. With regard to *I. suffruticosa* poisoning, it is interesting to note that hemolytic anemia has been reported in children who accidentally ingest an aniline-type of pigment (present in the diapers) used for laundry purposes; *I. suffruticosa* contains such a pigment.

Acute tubular necrosis occurs by two mechanisms: ischemic and toxic. The latter is the most probable explanation for the renal lesions observed in the calf of this report. A frequent set of events leading to ischemic tubular necrosis occurs in hypoperfused kidneys complicated by hemoglobinuria or myoglobinuria. Hemoglobinuria accompanies episodes of severe intravascular hemolysis and hemoglobinemia. Myoglobinuria accompanies acute rhabdomyolysis, as in azoturia of horses, capture myopathy in exotic or wild animals, or severe trauma. In these disease states, elevated serum concentrations of hemoglobin or myoglobin pass into the glomerular filtrate and accumulate in renal tubules causing hemoglobinuric or myoglobinuric nephrosis. Hemoglobin or myoglobin themselves are not toxic, in that intravenous infusions of these compounds into healthy animals produce no recognizable lesions. However stromal components of erythrocytes may be toxic and hemoglobin may enhance the tubular necrosis associated with ischemia. The renal cortices of animals with severe hemoglobinuria or myoglobinuria are diffusely stained red-brown or blue-black and often have intratubular heme casts that appear as red-black stippling on the external surface and continue into the cortex as radial oriented red streaks. The medulla may be stained dark red or contain red streaks. Microscopically, tubular lumina contain an abundance of orange-red, granular, refractile material, the characteristic appearance of a heme compound. Cellular swelling and pigmentation of tubular epithelial cells may be seen in association with increased serum concentrations of bilirubin, especially due to obstructive jaundice. The "hyalin droplets", that are observed histologically, are lysosomes distended by protein being degraded to return to the circulation as amino acids. The presence of hyalin droplets indicates that the process of protein reabsorption by the tubular cells is saturated.

The liver lesions in the *I. suffruticosa* poisoning in calves, although induced by the anoxia provoked by the hemolytic anemia should be differentiated from those produced by primarily hepatotoxic plants.

AFIP Diagnoses: 1. Kidney: Tubular epithelial degeneration and necrosis, diffuse, moderate, with tubular proteinosis and red-brown casts (hemoglobin), mixed breed, bovine.
2. Liver: Hepatocellular vacuolar degeneration (fatty change), centrilobular to midzonal, diffuse, moderate, with multifocal necrosis and bile stasis.

Conference Comment: The contributor has provide an excellent review of this entity, its' proposed pathogenesis, and related conditions. Although conference participants were unfamiliar with this particular condition, they agreed the histologic lesions were consistent with a hemolytic abnormality most likely due to a toxicological insult.

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