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503 Robert Grant Avenue
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CASE I - A02-335 (AFIP 2890560)

Signalment: 3 year-old, female, Rhesus macaque (*Macaca mulatta*), nonhuman primate.

History: This monkey was inoculated with SIVmac316 in June of 2001. In June of 2002, she was incoordinated and febrile and had dermatitis. Two days later she was bloated and had diarrhea. She was euthanized due to a poor response to treatment and a poor prognosis.

Gross Pathology: The mucosa of the stomach, small intestine and large intestine contains multiple red, raised, pedunculated, nodular masses (Fig. 1) ranging from 0.6-1.2 cm. The ileum and cecum are the most severely affected. There is moderate to marked enlargement of peripheral and mesenteric lymph nodes, with marked edema of the mesenteric lymph nodes. The spleen is enlarged and there is prominent lymphoid hyperplasia. There is mild ventral subcutaneous edema and a small amount (20 mls) of clear straw colored fluid in the abdomen. There is a 3-4 cm focus of thickened, crusty skin in the right inguinal region, and a similar 2-3 cm focus lateral to the right eye. There is marked, multifocal to coalescent thickening of the meninges, particularly along meningeal vessels, due to the accumulation of thick, green/yellow suppurative material. The mandibular and parotid salivary glands are markedly enlarged and there are multiple small (< 1mm) red foci throughout the mandibular salivary glands.

Laboratory Results: There is sporadic positive immunohistochemical staining with anti-CMV antibody.

Contributor's Morphologic Diagnosis: Severe, multifocal, proliferative and suppurative colitis (inflammatory pseudotumors) with intranuclear and intracytoplasmic CMV inclusion bodies, superficial mucosal hemorrhage, and intralesional *Balantidium coli*.

Contributor's Comment: Simian cytomegalovirus (sCMV), like its human counterpart, causes few if any, symptoms in monkeys. However, in an immune-compensated or suppressed patient, such as during transplantation, or as in this case, simian immunosuppressive virus-induced AIDs, CMV infection can be fatal[1]. The common identifiable gross lesions can be found in the meninges, eye, lung, heart, intestine, testicle and skin. Microscopically, lesions can be found in organs of the central and peripheral nervous, lymphatic, vascular, digestive and reproductive systems. It was thought that the infected cells were of mesenchymal origin, but recent work in clinical isolates of human CMV has shown that the virus is also endothelial cell-tropic and leukocyte-tropic[2]. The histological hallmark of the lesion induced by the virus is intranuclear and intracytoplasmic inclusion bodies and cytomegaly[1]. Furthermore, since CMV infection is known to induce the host cells to express proinflammatory proteins, such as IL-8 and RANTES, and also other binding molecules, such as ICAM-1 and LFA-3, recruitment and aggregation of neutrophils at the site of the lesion is very common and characteristic of a CMV infection[3].

Inflammatory pseudotumor, or IPT, is a quasineoplastic lesion that has been found to occur in nearly every site in the body. In humans, it is most commonly found in the orbit and lung. Due to its gross and radiographic resemblance to a neoplastic mass, it has been an area of focus for human medicine so as to avoid unnecessary radical surgery[4]. In the case presented here, the quasineoplastic lesion, as seen grossly and microscopically, is the result of an influx of neutrophils to the site of infection and proliferation of the mucosal epithelium. In most cases, both humans and nonhuman primates, CMV infections of the intestines are erosive, resulting in enterocolitis, hemorrhage, or intestinal perforation. Inflammatory mass formation is rare[5].

AFIP Diagnoses:

1. Colon: Colitis, proliferative, neutrophilic, acute, multifocal, moderate, with superficial mucosal hemorrhage, cytomegaly, and eosinophilic to basophilic intranuclear inclusion bodies, rhesus macaque (*Macaca mulatta*), nonhuman primate.
2. Colon: Intraglandular ciliated protozoa, numerous.
3. Colon: Intraglandular epithelial-attached bacilli.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant for veterinary parasitology. Conference attendees discussed the presence of surface associated spirochetes in glandular epithelium, consistent with *Brachyspira pilosicoli*. Although the significance in this case is unknown, the incidence of intestinal spirochetosis in clinically normal rhesus macaques was 42% in one study.[6]

Cytomegaloviruses are classified in the subfamily *Betaherpesvirinae* and are highly host-specific, causing low-grade, inapparent infections in immunocompetent humans, nonhuman primates, pigs, mice, and guinea pigs, among other species. Other betaherpesviruses of veterinary importance include porcine herpesvirus-2 (inclusion body rhinitis) and caviid herpesvirus-1 (guinea pig cytomegalovirus).[9]

Inclusion body rhinitis is a disease of young piglets causing necrosuppurative rhinitis with intranuclear inclusion bodies and cytomegaly in nasal mucous, harderian, and lacrimal glands, and renal tubular epithelium. Immunosuppressed piglets may develop systemic infection, causing widespread petechiae and edema.[7] Guinea pig cytomegalovirus causes karyomegaly and intranuclear inclusion bodies in the salivary gland, although interstitial pneumonia and multifocal necrosis in the lymph nodes, spleen, liver, and kidney may also be present. Guinea pigs are used as models for human cytomegalovirus, especially congenital infections. Like humans, the guinea pig placenta is hemochorial with a single layer of trophoblasts separating maternal and fetal circulation, facilitating transplacental transmission.[8,9]

Balantidium coli is a natural inhabitant of the intestinal lumen of pigs, rodents, and primates. Although usually an incidental finding, the organism rarely invades the mucosa and can cause enteritis and colitis. The trophozoites are large (50-200um), with a macronucleus and ciliated periphery.[10,11]

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CASE II - 02154-6 (AFIP 2897019)

Signalment: 16-year-old, male, Poodle, Dog (*Canis familiaris*).

History: Oral bleeding for one week.

Gross Pathology: Oral mass (3 x 2 cm) on the lingual frenulum.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Lingual frenulum: Pleomorphic liposarcoma.

Contributor's Comment: A moderately infiltrative mass, 1 cm in diameter, was present in connective tissue covered by a squamous epithelium (not present on all the sections); the epithelium was ulcerated, which explains the oral bleeding (on the slides, the ulceration seems to be accentuated by the surgery). The tumor was composed of polygonal cells arranged in sheets with little stroma. Tumor cells have indistinct borders and are moderately pleomorphic; some cells are rather small, with eosinophilic cytoplasm; other cells have abundant cytoplasm, filled with either numerous small vacuoles, or a single large vacuole. Anisokaryosis is focally severe and mitotic figures are present (Fig. 1). Small necrotic foci and discrete lymphoplasmacytic infiltrates are present.

Oil red-O staining of frozen sections demonstrates that the vacuoles are lipid droplets (Fig. 2), which helps to identify the tumor cells as lipoblasts. Most of the cells have numerous, small, lipid droplets, thus we diagnosed a pleomorphic liposarcoma[1].

The differential diagnosis includes infiltrative lipomas, composed of mature lipocytes which invade surrounding tissues[2], anaplastic carcinomas and balloon-cell melanomas[3,4]. Immunostaining with an anti-human cytokeratin (MNF116, Dako), reacts with a great majority of carcinomas, and was positive in the oral epithelium, but negative in the tumor cells (Fig. 3); the vacuoles did not stain by Periodic-Acid Schiff (Fig. 4). No cytoplasmic granules were observed with Schmorl staining (Fig. 5).

Oral liposarcomas are rare in human pathology[5] and only a small proportion develop in the tongue[6,7]. They generally occur in old patients[6-10], have a high recurrence rate

and almost no tendency for metastasis[6,8]. They are generally small (less than 3 cm in greatest diameter)[6,10]. Two histological types of lingual liposarcomas are described: well-differentiated[6,8,9] and myxoid[5,7]. Human lingual liposarcomas have low mitotic activity and a high recurrence rate with a long period between the first presentation and the first recurrence[6].

To our knowledge, lingual liposarcomas have never been described in veterinary pathology[11]. In dogs, liposarcomas are rare[12] and mainly described in the subcutis, thoracic and abdominal cavities[13]. One case was associated with a foreign body[14]. The tumor of this dog did not contain a foreign body. It seemed to exhibit a higher mitotic rate than its human counterparts. However, 8 months later, the dog is still alive and free of recurrence.

Liposarcomas of the tongue are possibly underdiagnosed in veterinary pathology. There is a risk of recurrence, with a long delay. Wide excision is recommended.

AFIP Diagnosis: Tongue: Liposarcoma, poodle, canine.

Conference Comment: Most conference attendees identified the tissue simply as mucous membrane and underlying connective tissue. In addition to the differential diagnoses mentioned by the contributor, attendees who favored conjunctiva as the tissue site also included meibomian gland carcinoma as a possible diagnosis.

Pleomorphic liposarcomas may resemble pleomorphic malignant fibrous histiocytomas (MFH); however, unlike MFH, liposarcomas have little to no collagenous stroma. Pleomorphic MFHs are composed of spindle cells with variable morphology and are arranged in a storiform pattern, whereas pleomorphic liposarcomas have highly variable morphology, varied cellular arrangement, and intracytoplasmic fat vacuoles in a small percentage of cells.[15]

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CASE III - AO 40673 (AFIP 2734406)

Signalment: 3-month-old male Simmental-cross calf, *Bos Taurus*.

History: This calf was a member of a small group of cows and calves. This calf was the only calf showing clinical signs. Labored breathing was noticed in the evening and the calf was dead the next morning. Dams were vaccinated with IBR, BVD, PI3 and BRSV vaccines.

Gross Pathology: At necropsy, there was pneumothorax and marked pulmonary emphysema. Numerous large subpleural bullae were present throughout the lungs. Overall, the lungs were heavy and wet. The ventral portions of the middle lung lobes were dark purple and consolidated.

Laboratory Results: Fluorescent antibody tests for IBR, BVDV, and PI3 were negative and positive for BRSV.

Contributor's Morphologic Diagnoses:

1. Subacute fibrinopurulent bronchopneumonia with bronchiolitis, syncytial cells and intracytoplasmic inclusion bodies.
2. Diffuse pulmonary emphysema and pneumothorax.
(Bovine respiratory syncytial virus pneumonia)

Contributor's Comment: In sections of lung, bronchi and bronchioles are filled with degenerate neutrophils, erythrocytes and cell debris. There is necrosis of bronchiolar epithelium and numerous syncytial cells are present in the lumens. Pleomorphic eosinophilic intracytoplasmic inclusions are present in bronchiolar epithelial cells and in syncytial cells. Peribronchiolar alveoli are filled with degenerate neutrophils, erythrocytes, and fibrin. The lungs are markedly congested and septal lymphatics are filled with fibrinocellular exudate. Septal edema and emphysema are marked.

Respiratory syncytial viruses are pneumoviruses in the family Paramyxoviridae. Members of the genus *Pneumovirus* include human, bovine, ovine, and caprine respiratory syncytial viruses, pneumovirus of mice, and turkey rhinotracheitis virus. BRSV is distributed worldwide and antibody prevalence in the United States ranges from 65-81%. Clinical disease is most common in calves less than 6-months of age and is more severe if infections are concurrent with other respiratory viruses or bacterial agents. Secondary bacterial pneumonia is common. Consistent findings include emphysema, severe necrotizing bronchiolitis with syncytial cells, purulent and bronchointerstital pneumonia. Inclusion bodies are evident in the early stages of infection. Bronchiolitis obliterans is a common sequela. Diagnosis is confirmed by fluorescent antibody test or immunohistochemistry. Virus isolation is difficult.[1,2]

The present calf died suddenly due to pneumothorax secondary to severe pulmonary emphysema. At necropsy, atypical interstitial pneumonia was considered in the differential diagnosis. Microscopically, inclusion bodies and syncytial cells were prominent in bronchiolar epithelium and the diagnosis was confirmed by immunofluorescent antibody staining. Bacterial colonies were present in some sections.

AFIP Diagnoses:
1. Lung: Pneumonia, bronchointerstitial, neutrophilic, acute, diffuse, severe, with necrosis, syncytia, and eosinophilic intractyoplasmic inclusion bodies, Simmental-cross, bovine.
2. Lung: Rare epithelial basophilic intranuclear inclusion bodies.

Conference Comment: Basophilic intranuclear inclusion bodies, consistent with adenoviral inclusions, were identified in the respiratory epithelium. In addition to bovine respiratory syncytial virus (BRSV), conference attendees discussed other causes of bovine bronchointerstitial pneumonia, including parainfluenza type-3 (PI-3) and bovine adenovirus.

Bovine respiratory syncytial virus is an important component of the bovine respiratory disease complex (BRD) that most often affects young cattle and predisposes to secondary infections, like *Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*. Bronchoconstriction is an important feature of BRSV that leads to airway obstruction and terminal interstitial emphysema. Evidence suggests that virus-infected cells activate complement, which causes mast cell degranulation and histamine-induced bronchoconstriction. Virus specific IgE antibody is also implicated in the pathogenesis and resultant clinical signs.[3,4,5]

Differential diagnoses for any bovine pneumonia should include those agents associated with the BRD complex. This includes enzootic pneumonia of calves (a variety of etiologic agents), pneumonic mannheimiosis (*Mannheimia haemolytica*), respiratory hemophilosis (*Haemophilus somnus*), infectious bovine rhinotracheitis (bovine herpesvirus-1), mycoplasmosis, and adenovirus.[6]

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CASE IV - 020584 and 0201878 (AFIP 2891625)

Signalment: Adolescent (1.7-3.8 kg) female Cynomolgus monkey(s), *Macaca fascicularis*.

History: Cynomolgus monkeys were exposed to spores of anthrax (Ames strain) by inhalation to determine the LD_{50} and pathology, in order to investigate this species as a

model for inhalation anthrax subsequent to the reduced availability of Rhesus monkeys for this purpose.

In general, monkeys remained bright and alert for several days (2-7), then became lethargic and non-responsive, with death (or euthanasia) within a couple hours.

Gross Pathology: The gross photographs represent tissues from 3 monkeys (Figs. A-C); slides provided were from two monkeys (one of which was in these gross photographs). The most common gross lesions were mild splenomegaly, lymph node enlargement, and hemorrhages in various organs, particularly involving the meninges and the lungs (Fig. D). Mediastinal hemorrhage and/or edema affected 29% of the monkeys.

[1]The gross brain image annotated with superscript 1 (Fig. C) is from Vasconcelos et al. Pathology of inhalation anthrax in cynomolgus monkeys (*Macaca fascicularis*). Laboratory Investigation 83(8):1201-1209, 2003.

Laboratory Results: *Bacillus anthracis* was cultured from blood of all affected animals (collected when moribund or as soon as possible post mortem). Bacteria in histologic sections were usually strongly Gram-positive.

Contributor's Morphologic Diagnoses:
SPLEEN (0200584):
1. Lymphocytolysis (necrosis and apoptosis), severe, acute, diffuse, with hemorrhage and numerous bacteria characteristic of *Bacillus anthracis*.
2. Vasculitis, moderate, acute, multifocal.

BRAIN (0201878):
1. Meningitis, hemorrhagic and suppurative, mild to moderate (based upon gross), acute, multifocal, with bacteria characteristic of *Bacillus anthracis*.

Contributor's Comment:
Description:
0200584 (Spleen): There is severe loss of lymphoid tissue from the periarteriolar lymphoid sheaths, which are almost obliterated, and from splenic corpuscles (Fig. 1). The mantle zones of the splenic corpuscles are hemorrhagic (Fig. 2). The splenic corpuscles contain abundant nuclear debris ("nuclear dust") both extracellularly (Fig. 3, green arrows) and within macrophages. Some macrophages contain brownish pigment (hemosiderin). Only occasional splenic corpuscles still contain intact lymphocytes. The red pulp contains numerous large square-ended ("boxcar"-like) rods (bacteria, black arrow in Fig. 3) and abundant granular eosinophilic material. Fewer bacteria are visible within arteries and veins, which have an increased number of leukocytes (predominantly neutrophils). Many sections have arteries in which leukocytes are lined up along the endothelium (pavementing) or are beneath the endothelium (Fig. 4; the vessel lumen is at lower left). In an occasional section there is fibrillar eosinophilic material within splenic corpuscles, interpreted to be fibrin.
Special stain (Hopps): Bacteria are Gram-positive.

0201878 (Brain): The meninges have foci of hemorrhage and infiltration (minimal to mild) with leukocytes (moderate numbers of neutrophils and fewer lymphocytes) (Fig. 5). Within these areas there are few to numerous square-ended rods (bacteria, Fig. 6 arrow), which are also present intravascularly although usually in smaller numbers.

Significance:

The recent use of anthrax as an agent of bioterrorism in the United States has resulted in increased use of monkeys as models for inhalation anthrax of humans. Rhesus monkeys are currently less available than Cynomolgus monkeys, which are a suggested alternative for investigations into the pathogenesis and immunology of this disease (1).

Pathogenesis:

The pathogenesis of inhalation anthrax is thought to begin with macrophage phagocytosis of inhaled spores. The macrophages migrate to intrathoracic (bronchial or mediastinal) lymph nodes and the spores germinate and are released. The vegetative bacilli release 2 exotoxins known to be important to the pathogenesis, lethal toxin (LT) and edema toxin (ET) (reviewed in 2). Lethal toxin is composed of lethal factor (LF) and protective antigen (PA), whereas edema toxin is composed of edema factor (EF) and protective antigen (PA). It is thought that PA diffuses more rapidly from the bacilli and binds cells, forming pores. Subsequent binding of lethal factor or edema factor forms the respective toxins, which enter the cell. Anthrax strains harboring mutations to either the LF or EF protein genes are less lethal than the parent strain, whereas mutations in the PA gene abolish lethality (reviewed in 2).

Lethal toxin is a zinc protease to which macrophages are particularly susceptible (4, reviewed in 2). It inhibits mitogen-activated protein kinase (MAPK) *in vitro* by proteolysis of the N-termini of mitogen-activated protein kinase kinases (MAPKK), rendering them incapable of activating MAPK (3, and reviewed in 2). Depletion of macrophages, pre-treatment with an IL-1 receptor antagonist, or passive immunization against IL-1 all render mice resistant to lethal toxin (4). Sublethal doses of LT may induce macrophages to produce TNF-alpha and IL-1 (4), which may account for the shock-like death.

Edema toxin is considered responsible for the edema characteristic of anthrax septicemia. It is an adenylate cyclase, converting ATP into cAMP in a Ca^{++}- and calmodulin- dependent manner (2). Edema toxin action upon macrophages inhibits phagocytosis and the oxidative burst (reviewed in 2).

Whereas herbivores are extremely susceptible and have minimal lesions, carnivores are resistant and often have lesions at the site of entry (e.g. pharyngitis). Primates are considered to be of intermediate susceptibility.

Comparative Pathology:

The gross and microscopic pathology of anthrax in both Rhesus monkeys and Cynomolgus monkeys is very similar to that in humans (1, 5-9). Lesions are typical of a fulminant septicemia. Grossly, hemorrhages are common in the meninges, lung, and mediastinum, although they can occur anywhere in the body (1, 5, 7). Enlargement of intrathoracic lymph nodes is characteristic (1, 5, 7). Mild splenomegaly appears more common in non-human primates than in humans (1, 5, 8, 9). Animals that die peracutely (i.e. within 2-3 days) have fewer lesions than those that survive several days. Occasional non-human primates exhibit a virtual absence of gross lesions (1, 6, 7), suggesting the possibility that human cases might not be diagnosed if histopathology or bacteriologic cultures were not performed.

Microscopically, hemorrhages are common in intrathoracic lymph nodes, lungs, meninges, adrenal glands, mediastinum, and gastrointestinal tract, possibly secondary to necrotizing vasculitis, which has been reported in all three species (1, 7-9). Pulmonary edema (as well as less-frequent acute pulmonary inflammation), hemorrhagic meningitis, and mild suppurative mediastinitis and lymphadenitis are all characteristic microscopic findings in primates including humans (1, 5-9). Marked lymphocytolysis (apoptosis and/or necrosis) in the spleen and lymph nodes is very common in all 3 species, with some differences among reports as to whether a B- or T-lymphocyte predilection exists (1, 7, 9). There do not appear to be reports of lymphocytolysis occurring in the thymus, although this may be a reflection of the age of patients and/or animals involved (1, 5-9).

AFIP Diagnoses:
1. Spleen: Lymphoid necrosis, diffuse, with perifollicular hemorrhage, fibrin, and myriad gram-positive bacilli, cynomolgus macaque (*Macaca fascicularis*), nonhuman primate.
2. Cerebellum, meninges: Meningitis, neutrophilic, acute, with vasculitis, and myriad bacilli.

Conference Comment: The contributor gives an excellent review of anthrax. Conference attendees noted that a few neutrophils extend beyond the affected meninges into the neuropil. Attendees also noted eosinophilic globules within the white matter, as noted by Vasconcelos, et al. and interpreted to be either necrotic oligodendroglia or macrophages.[1]

Herbivores are most susceptible to anthrax, primates have intermediate susceptibility, and carnivores and pigs are least susceptible. Under natural conditions, most birds, reptiles, and fish are resistant because their normal body temperature is outside of the optimal range for the anthrax bacillus. Ostriches are the only avian species reported to have been naturally infected with anthrax.[10,11,12]

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