**CONFERENCE 21**
20 March 2002

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CASE I – N60 (AFIP 2744071)

**Signalment:** 4-year-old, neutered female, Domestic Shorthair, Feline

**History:** A single small nodule was present for approximately a week on the right ear pinna.

**Gross Pathology:** 4 x 5 mm in diameter, ulcerated nodule on the ear pinna.

**Laboratory Results:** None. The entire nodule was submitted for histopathology.

**Contributor's Morphologic Diagnosis:** Skin: chronic, focal, granulomatous dermatitis with intrahistiocytic acid-fast bacteria.

**Contributor's Comment:** A well demarcated, non encapsulated, granulomatous inflammation is observed in the dermis, filling the entire space between the ear cartilage and surrounding epidermis. It is predominantly composed of reactive histiocytes with open-faced, peripherally located nuclei and abundant eosinophilic, occasionally vacuolated cytoplasm. Numerous Ziehl-Neelsen positive organisms are within the cytoplasm. Interspersed foci of neutrophilic polynuclear cells are present. The adnexa are effaced by the inflammation. The epidermis is raised, slightly hyperplastic and focally ulcerated.

The cellular characteristics of the nodule (dense sheets of reactive histiocytes in the absence of giant cells, the large number of acid fast bacteria in the cytoplasm of the histiocytes) are highly suggestive of feline leprosy. Feline leprosy is a rare skin disease caused by *Mycobacterium lepraemurium*, a non-culturable mycobacterium. Macroscopically this disease is characterized by the presence of single or multiple grouped nodules most frequently located on the head or the neck, more rarely on the trunk or the forelimb. The mode of transmission is

still unknown although arthropod vectors have been suspected due to the occurrence of the lesion following summer and occurring often in cats living in seaport cities.

**AFIP Diagnosis:** Haired skin: Dermatitis, nodular, granulomatous, focal, severe, with numerous intrahistiocytic bacilli, Domestic Shorthair, feline.

**Conference Comment:** Two distinct lesions are recognized with feline leprosy. The lepromatous form, which is characterized by diffuse sheets of epithelioid macrophages, scattered lymphocytes and plasma cells, and abundant intracellular acid fast bacilli, is associated with a poor cell-mediated response. In contrast, the tuberculoid form which is associated with a strong cell-mediated response, is characterized by central caseation and relatively fewer organisms. Demonstration of organisms can be accomplished using Ziehl-Neelsen or the Fite-Faraco modification. The absence of growth in routine cultures is often diagnostically helpful. The organism is extremely difficult to culture, requiring an enriched egg yolk medium.

Other mycobacterial dermatitides include classical tuberculosis (*M. bovis*, *M. tuberculosis*) and atypical mycobacteriosis caused by Runyun group IV mycobacteria (e.g. *M. fortuitum*, *M. chelonei*, *M. smegmatis*, *M. phlei*). Group IV mycobacteria are ubiquitous in nature, grow rapidly, and can result in opportunistic infections. Unlike feline leprosy, the inflammation of atypical mycobacteriosis is characterized as pyogranulomatous and the organisms are sparsely scattered, often located within clear vacuoles, and best demonstrated using the Fite-Faraco modification.

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**CASE II** – G-6010/00 (AFIP 2806496)

**Signalment:** Two-year-old, male, *Callithrix jacchus*

**History:** The animal was send to the German Primate Center for post mortem examination with a history of sudden severe depression and illness accompanied by erosive-ulcerative stomatitis. The animal died within two days after developing first symptoms. Several other animals of the group were affected and died.

**Gross Pathology:** The animal exhibited an acute erosive-ulcerative gingival-stomatitis. The tongue was covered with multifocal individual to coalescing, up to 0.5 to 1.0 cm diameter lingual erosions with rough borders. Some of these lesions progressed to real lingual or gingival ulcers covered by a fibrinopurulent exudate. No other gross lesions were seen except a mild splenomegaly and severe lymphadenopathy of the regional lymph nodes.

**Laboratory Results:** On bacteriologic examination *Streptococcus* species and *Neisseria* species were isolated from the tongue. Parasitology revealed no pathogens. Hematology revealed a moderate leucocytosis.

Immunohistochemistry using a monoclonal antibody directed against *Herpes simplex* virus types I and II antigen (Biogenex, Cat. No., MU086-UC) revealed a specific antigen-antibody reaction in the lingual lesions. The whole ulcer region and in particular the degenerated epithelial cells contained a high number of viral particles visualized with this strongly specific antigen-antibody reaction. Electron microscopic examination demonstrated the presence of intranuclear viral nucleocapsids either empty or filled with electron dense material as well as large numbers of enveloped virions 140 nm in diameter in the cytoplasm and intercellular spaces. The size, location and morphology were consistent with identification of the virus as a member of the herpes virus family.

Herpes virus was isolated from swab specimens of the oral cavity of the animal. Growth characteristics of the isolated virus were consistent with those of alpha-herpes viruses. To identify the virus, PCR analysis was performed on several specimens obtained at necropsy and frozen by $-80°C$. The results demonstrated the presence of *Herpes simplex* type 1.

**Contributor's Morphologic Diagnosis:** Tongue, stomatitis, ulcerative, herpetic. *Herpes simplex* was isolated as causative agent.

**Contributor's Comment:**  Histology revealed severe vesiculation and ulceration of the squamous epithelia of the tongue with acantholysis, parakeratosis, coagulation necrosis, and polykaryocytosis forming necrotic plaques extending from the submucosa to the surface.  Epithelial cells located at the ulcer margins showed various stages of degeneration and necrosis.  Intranuclear inclusion bodies were seen particularly in border areas of the vesicles and ulcers.  These inclusions filled the nucleus or were surrounded by a clear halo.  A marked neutrophilic and lymphohistiocytic infiltration was evident in the underlying submucosa.  All other organs investigated were without significant findings.

Nonhuman primates are primary hosts to quite a number of herpesviruses.  The herpes virus family consists of three subfamilies: alpha-herpes virus (simplex viruses), beta-herpes viruses (cytomegaloviruses) and gamma-herpesviruses (lymphotrophic herpesvirus).  The human *Herpes simplex* viruses type 1 and 2 (HSV-1 and HSV-2), the *Cercopithicine herpesviruses* 1 (*Herpesvirus simiae,* B-virus), 2 (simian agent 8, SA8) and 16 (*Herpesvirus papio*, HVP-2) are members of the alpha-herpesvirus subfamily.  The B-virus is specific for macaques (*Macaca sp.*), SA8 occurs in African green monkeys (*Cercopithecus aethiops*) and HVP-2 in baboons (*Papio* sp.).  These viruses generally cause mild or inapparent infections in their natural host, several have been associated with severe infections when transmitted to other species.  The well-known spontaneous interspecies transmissibility of herpesviruses may result in fatal diseases of either man or animals.

*Herpes simplex* virus belongs to one of the better known human to nonhuman primate transmissible viruses, but spontaneous infections in monkeys appear to be rare.  The original or reservoir hosts are humans.  Clinical symptoms of this generalized febrile disease are described as acute gingival-stomatitis during the primary infection.  Recurring oral or labial vesicles *(H. simplex* type 1) as well as genital vesicles and ulcers (*H. simplex* type 2) in adults represent reactivation of latent herpes infection with virus shedding.

The most characteristic gross findings in natural and experimental *Herpes simplex* infection are discrete vesicles, necrotic plaques, erosions or ulcers on the mucous membranes and the mucocutaneous junction of the lips affecting the entire oral cavity or extending into pharynx and esophagus.

Histopathological findings are characterized by increased eosinophilia of affected cells, which become ballooned and then necrotic with karyolytic or karyorrhectic nuclei.  This lesion progresses to vesicles and necrotic plaques extending from the surface to the submucosa.  The necrotic tissue sloughs leaving ulcers.  Adjacent to necrotic foci, ballooned epithelial cells containing intranuclear inclusion bodies are evident.  The underlying tissues are infiltrated with neutrophils and mononuclear cells.

*Herpes simplex* infection is not common in any of the primate species. Susceptibility to *Herpes simplex* seems to vary among different primate species. Many people suffer from periodic recurrence of the lesion often with several episodes each year. Monkeys most likely become infected by humans, who can excrete the virus during the acute phase of the disease and even in the absence of visible lesions. The routes of transmission from man to nonhuman primates are unknown, the assumption of contact or aerogenous infections even in those interspecies transmission seems to be reasonable. Once present in a colony, the disease spreads rapidly with very high morbidity and mortality.

Since humans are the natural or reservoir host for the virus the use of appropriate protective clothing and face masks for humans handling nonhuman primates should greatly reduce the risk for infection.

The diagnosis depends on the histological demonstration of typical intranuclear herpesvirus inclusion bodies and the ultrastructural demonstration of typical herpesvirus particles. Immunohistochemistry can help differentiate the various herpes viruses. The virus can be cultured, isolated and identified.

**AFIP Diagnosis:** Tongue: Glossitis, necro-ulcerative, subacute, multifocal, moderate, with epithelial syncytia and intranuclear inclusion bodies, etiology consistent with herpesvirus, Common Marmoset (*Callithrix jacchus*), nonhuman primate.

**Conference Comment:** The contributor has provided a concise review of this condition. Conference participants favored an etiological diagnosis of alphaherpesviral glossitis with a differential diagnosis that included *Herpesvirus tamarinus* (Cebid herpesvirus 1, herpes T), *Herpesvirus simiae* (Cercopithecine herpesvirus 1, herpes B), and *Herpesvirus hominis* (Herpes simplex). Within susceptible species, lesions occurring as a result of infection by these viruses can appear histologically identical.

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**CASE III** – 12650-99 (AFIP 2787172)

**Signalment:** 2-year & 9-month-old, male, Basenji mix (*Canis familiaris*), canine.

**History:** This dog was acquired as a 2-month-old puppy by his owner, a missionary, in Cameroon (west-central Africa). He "immigrated" to the USA at 9-months of age. During an otherwise routine orchiectomy procedure, the referring veterinarian reported a large number of worm-like parasites falling out of the incision site through the scrotum. Other parasites were palpable in the subcutis along the caudal aspect of the abdomen near the scrotum. Abdominal radiographs and ultrasound revealed widespread nodules in the liver, spleen, mesentery, and abdominal wall. No specific therapy was initiated. Two years later, ascites and liver failure developed so euthanasia was performed.

**Gross Pathology:** At necropsy, parasite infestation was widespread. Innumerable encysted parasites were disseminated throughout the liver, mesentery, and intestinal serosa. Encysted parasites were also scattered in the kidneys, spleen, diaphragm, abdominal wall, lungs, pericardium, and cerebral meninges. These cysts were discoid, approximately 0.5 cm in diameter, and had a translucent wall through which "C"-shaped highly annulated parasites were visible. Also within the peritoneal cavity was a fibrous yellow-white-gray mass within which were encysted parasites and which encased and strangulated segments of the intestine, resulting in perforation and abscess formation.

**Laboratory Results:** None.

**Contributor's Morphologic Diagnosis:** Liver: Encysted pentastome nymphs with mild hepatocellular atrophy, multifocal inflammation, and canalicular cholestasis.

**Contributor's Comment:** Pentastome nymphs had morphologic features consistent with *Armillifer* sp. Characteristics of pentastome include a nonsegmented body, metamerically arranged striated musculature, chitinous cuticle with numerous glands, acidophilic glands that surround the intestine, and a "sclerotized" mouth opening flanked by two pairs of large hooks; they lack a circulatory or a respiratory system. Nymphs of *Porocephalus* can be distinguished from those of *Armillifer* by the accessory external mouth hook in *Porocephalus*. Mammals serve as the intermediate host for the nymph stage of *Armillifer* sp. The definitive hosts are snakes in which adult pentastomes parasitize the respiratory tract or body cavity.

**AFIP Diagnosis:** Liver: Pentastome nymphs, many, with multifocal mild lymphocytic inflammation, biliary hyperplasia, and cholestasis, Basenji mix, canine.

**Conference Comment:**  Pentastomiasis was first described by the French veterinary surgeon Chabert in 1787.  The pentastome mouth, which is surrounded by a chitinous ring and four large hooks, lead early researchers to believe it had five mouths and hence the name.  While most researchers place pentastomes in their own phylum (Pentastomida), various authors have suggested inclusion with crustaceans, arachnids, or annelids.

A unique feature of pentastomes is the presence of numerous sclerotized openings.  Present within the body wall, sclerotized openings are rings that appear deeply eosinophilic when stained by hematoxylin and eosin or black when stained by Movat pentachrome.  Sclerotized openings, which can be seen in the molted cuticles surrounding nymphs, are associated with cuticle production.

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**CASE IV** – 992206-31 (AFIP 2758056)

**Signalment:**  Adult, male, rhesus monkey (*Macaca mulatta*)

**History:**  This rhesus monkey was involved in a vaccine efficacy study.  It received a lethal dose of anthrax (*Bacillus anthracis*) spores by aerosol. He was found dead on day 4 post-exposure.

**Gross Pathology:**  Mediastinal lymph nodes: hyperemic; lungs: congested; brain: hemorrhagic meningitis.

**Laboratory Results:**  Not available.

**Contributor's Morphologic Diagnosis:**  Brain:  Meningitis, hemorrhagic, acute, multifocal, moderate with multifocal encephalitis and myriad bacilli.

Etiology:  *Bacillus anthracis*

**Contributor's Comment:**  Anthrax is an infection caused by the gram-positive, aerobic, spore forming bacterium, *Bacillus anthracis*.  The inhalation form, although rare, is nearly always fatal due to the rapid progression of the disease with few clinical signs and symptoms until the terminal stages of the disease.  This form of the disease, so-called "woolsorter's disease" or "ragpicker's disease", was one of the first occupational pulmonary diseases identified in humans.  Observations made in human and experimental cases of inhalation anthrax suggest that the lung parenchyma serves as a portal of entry for the organisms, which results in a generalized systemic disease rather than a primary pneumonic disease.  Inhaled spores are phagocytosed by macrophages and carried by lymphatics to local mediastinal lymph nodes, where bacilli germinate and grow.  Septicemia appears to occur when the phagocytic capacity of the lymph node's sinus histiocytes becomes overwhelmed.

Hemorrhagic meningitis with variably intense neutrophilic inflammatory infiltrates is frequently associated with inhalation anthrax in both humans and nonhuman primates.  It has been suggested that the degree of leukocytic response may be related to relative host susceptibility, with highly susceptible hosts developing a mild response, in contrast to the more intense response seen in hosts resistant to anthrax.

To date, there have been two papers describing detailed pathologic findings in the brain of nonhuman primates following inhalation exposure to anthrax.  In the first paper, Gleiser et al. reported pathologic findings in rhesus monkeys exposed to either a high or a low infectious dose of anthrax spores.  The incidence of hemorrhagic meningitis in their high-dose group was 33%.  These authors emphasized that in their study, hemorrhage in the brain was always confined to the meninges or within Virchow-Robin (VR) space.  In the second paper, our laboratory reported pathologic findings in rhesus monkeys exposed by aerosol to a high dose of anthrax spores.  In contrast to Gleiser's findings, histopathologic evidence of hemorrhagic meningitis in 7 of 13 (54%) monkeys in our study exceeded the incidence in Gleiser's monkeys despite the use of comparable infectious doses, but different strains of anthrax.  The hemorrhage in the brains of monkeys in our study was not always confined to the meninges or VR spaces.

In a 1993 paper, Abramova et al. reported pathologic findings in 42 human deaths from inhalation anthrax in the former Soviet Union.  That 1979 incident in Sverdlovsk, U.S.S.R. is said to be the largest documented outbreak of human inhalation anthrax.  At autopsy, examination of the brains from 40 those victims (2 brains were unavailable for examination), hemorrhagic meningitis was found in 21, an incidence of 52.5%.  This high incidence might be due in part to prolonged survival times of these patients.  The incidence in that outbreak compares to the relatively high incidence in the two nonhuman primate reports mentioned above.

Qualitatively, pathologic changes in the CNS of primates were almost identical with those in humans.  Grossly, meningeal hemorrhages were more prominent on the dorsal aspect of the brain, giving the appearance of a "cardinal's cap", characteristic of anthrax meningitis.  Microscopically, hemorrhage in the neuropil was also found in some Sverdlovsk patients as it was in our study.

In this case we are submitting for WSC, the following features are present multifocally: in the meninges, there is a mild to moderate infiltrate of PMN and macrophages, scattered hemorrhage and fibrin, myriad bacilli, and acute necrotizing vasculitis or fibrinoid necrosis of meningeal vessels.  In the cerebrum, there is a minimal infiltrate of PMN directly subjacent to the meninges, multifocal mild hemorrhage in Virchow-Robin spaces or in the surrounding neuropil, and within blood vessels there is leukocytosis and endothelial cell hyperplasia/hypertrophy.  In most rhesus monkeys similarly infected, the inflammatory cell infiltrate would be less intense, but otherwise this case has the typical features of anthrax meningitis.  Transmission electron microscopy of a mediastinal lymph node demonstrated degraded bacilli within a macrophage. This tissue was immunoreacted with anti-polysaccharide antibody which labels the bacterial cell wall.  Since bacterial capsular material is usually lost following routine processing for TEM, the anti-polysaccharide antibody works better in this application than anti-capsule.

**AFIP Diagnosis:**  Cerebrum:  Meningitis, suppurative, acute, diffuse, moderate, with necrotizing vasculitis and numerous bacilli, Rhesus Macaque (*Macaca mulatta*), nonhuman primate.

**Conference Comment:**  The major virulence factors of anthrax include a glutamic acid capsule and an exotoxin composed of three distinct proteins: protective antigen (PA), lethal factor (LF), and edema factor (EF).

Protective antigen is the membrane-translocating component of the toxin and works synergistically with LF forming lethal toxin (LT) and with EF forming edema toxin (ET).  As PA binds to cell surface receptors it is cleaved by cell surface proteases generating PA63.  PA63 possess high affinity binding sites for both LF and EF.  The complex is internalized via receptor mediated endocytosis forming a

voltage gated channel. This allows the translocation of LF and EF into the cytosol where they exert their toxic effects.

Lethal toxin is a zinc endopeptidase that in high concentrations is cytolytic for macrophages. Previously, low or sublytic doses of LT were thought to induce expression of interleukin-1 (IL-1) and tumor necrosis factor alpha (TNFa). IL-1 and TNFa decrease expression of thrombomodulin and increase endothelial cell selectin expression inhibiting normal clotting control and inducing a procoagulant state. In contrast, more recent literature suggests that at low or sublytic doses, LT cleaves mitogen-activated protein kinase kinases (MAPKKs) Mek1 and Mek2 and MKK3. As a result there is substantial inhibition of signaling pathways and cytokine messenger RNA transcription. There is decreased production of nitric oxide and TNFa during the initial inflammatory response. This muted inflammatory response benefits the invading organism during the first stages of infection.

Edema toxin has calcium and calmodulin-dependent adenylate cyclase activity. ET raises cellular cAMP causing electrolyte and fluid loss, inhibition of neutrophil phagocytosis, and induction of IL-6 secretion by monocytes.

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