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Department of Veterinary Pathology
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Conference Moderator: COL Kelly Davis
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US Army Medical Research Institute of Infectious Disease
Ft. Detrick, MD 21702

CASE I – 99-2038 (AFIP 2694761)

Signalment: 2-year-old, Quarter Horse, female, equine, equus caballus

History: This filly was purchased in December 1998 in Texas and transported to Kansas. She was presented in February with head pressing, loss of coordination, hypermetria, wide-based stance, and lethargy. The mare was euthanized and necropsied. She had been vaccinated with a killed vaccine against Eastern equine encephalomyelitis a week prior to the onset of the clinical signs.

Gross Pathology: No significant gross lesions were found in the central nervous system.

Laboratory Results: FA test for rabies was negative. Virus isolation was suggestive of Equine encephalitis virus. Negative staining electron microscopy of the isolate by the National Veterinary Services Laboratory (NVSL) at Ames, Iowa revealed that the isolate was a togavirus and compatible with one of the equine encephalomyelitis viruses. The final identification of the virus was eastern equine encephalomyelitis virus (EEEV) via complement fixation test at NVSL.

Contributor's Morphologic Diagnosis: Brain: encephalitis, nonsuppurative, moderate to severe, diffuse with gliosis, multifocal necrosis and focal perivascular hemorrhages.

Etiology: Eastern equine encephalomyelitis virus, a virus belonging to the genus *Alphavirus* in the family Togaviridae.

Contributor's Comment: Histologic changes are widespread in the cerebrum, cerebellum, midbrain and brain stem, being most prominent and extensive in the

cerebrum. Lesions in the brain consist of widespread areas of perivascular lymphomonocytic cuffing, focal areas of necrosis, hemorrhage, and gliosis. Occasional neutrophils are mixed with the perivascular cuffs or with glial cells in the neuropil. Neuronal changes are not prominent but there does appear to be some satellitosis, occasional suggestion of neuronophagia and fibrinoid necrosis of occasional venules. There is focal to locally extensive necrosis, predominantly of white matter, with lymphocytes, a few neutrophils, and cells with prominent karyorrhexis in necrotic foci in the cerebral and cerebellar cortex.

Eastern equine encephalitis virus causes a disease that results in severe CNS dysfunction and often death in horses, human beings, and other incidental hosts, including commercially raised poultry, emus, and swine. Outbreaks of the disease attributable to EEEV are generally located along the Atlantic coastal region of the United States and along the Gulf Coast from Florida to Texas. Outbreaks of disease have been reported in inland regions of the United States including the upstate region of New York, Ohio, Indiana, Michigan, Wisconsin, and South Dakota. The EEEV life cycle involves a vector-host system between mosquitoes and wild birds. Outbreaks of disease attributable to EEEV are expected to develop from mid- to late summer. The current case presents peculiar circumstances, i.e. a recently vaccinated horse in the middle of the winter in a geographical location where the disease seldom occurs.

Specific environmental conditions that precipitate disease outbreaks attributable to EEEV have not been established, although outbreaks usually are associated with hot, excessively rainy weather that creates ideal conditions for increases in mosquito populations. Some investigators have postulated that outbreaks may have resulted after infected mosquitoes were brought into an area by the wind.

Mechanisms of virus maintenance in regions in which the life cycle of the primary EEEV vectors is interrupted during the winter season are unknown. Potential mechanisms that allow the virus to persist in these areas include transovarial transmission of virus, overwinter survival of infected adult mosquitoes, chronic latent infections in birds, and overwinter survival of virus in other vertebrate hosts such as reptiles and snakes. How this filly became infected is still unclear.

AFIP Diagnosis: Cerebrum: Meningoencephalitis, nonsuppurative, diffuse, moderate, with multifocal necrosis and gliosis, Quarter Horse, equine.

Conference Comment: Eastern equine encephalitis virus (EEEV) is a single stranded, positive sense, RNA virus of the alphavirus family. The virus actively replicates in the mosquito and is transmitted to the horse which is considered a dead end host. In North America the primary natural reservoir of EEEV is believed to be wild birds. A recent study in horses identified EEEV in cells of the heart, lung, kidney, spleen, stomach, intestine, urinary bladder, adrenal glands, and CNS. CNS lesions were described in the cerebral cortex, thalamus, hypothalamus, mesencephalon, cerebellum, and spinal cord. Viral antigen was identified in neurons, astrocytes, oligodendrocytes, and microglial cells.

In this case, additional diagnostics were performed at the United States Army Medical Research Institute of Infectious Disease (USAMRIID). By immunohistochemistry, the section was positive in multiple cell types for viral antigen using a pooled polyclonal alphavirus antibody. By in situ hybridization, neurons and axonal processes were positive for viral RNA using an EEEV specific probe.

Additional viral encephalitides were discussed in conference and include: West Nile virus (flavivirus), Rabies virus (rhabdovirus), Equine herpesvirus 1, and Japanese encephalitis virus (flavivirus). Viral encephalitides are often characterized by varying degrees of perivascular cuffing and leukocytic expansion of Virchow-Robin space. Characteristic lesions (e.g. vasculitis with EHV-1), distribution (e.g. lesions generally confined to the midbrain, brainstem, and spinal cord with West Nile virus), or structures (e.g. Negri bodies with Rabies virus) are diagnostically helpful.

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CASE II – 010591-1 (AFIP 2788025)

Signalment: 9-year-old, male, domestic long-haired cat

History: This cat was presented to the veterinary medical teaching hospital with a two weeks history of lethargy and abdominal distension. Upon physical examination, the cat showed an abdominal effusion. He died one week after admission.

Gross Pathology: At necropsy, all abdominal serosal surfaces were covered by a black granular substance. The liver contained numerous yellow to black necrotic foci scattered throughout the parenchyma. The abdominal cavity contained excessive fluid which was yellow and slightly turbid.

Laboratory Results: The leukogram revealed a marked leucocytosis (70,488/ul) due to a neutrophilia (51,264/ul) and a regenerative left shift.

Ultrasonographic examination of the liver showed several disseminated hypoechogenic areas and confirmed the abdominal effusion.

Fine needle aspirates of the liver showed a large number of pigmented fungal hyphae, degenerate neutrophils and large number of necrotic cells.

The abdominal fluid was classified as a modified transudate, based on total nucleated cell count (1,080/ ul) and total protein content (29g/l). It contained a mixture of non-degenerate neutrophils, mesothelial/macrophages cell types and some lymphocytes. Sparse pigmented fungi were found in macrophages and extracellularly.

The cat was serologically negative for feline leukemia virus, feline immunodeficiency virus, and feline infectious peritonitis virus.

Cultures of various organs yielded a fungal organism identified as *Cladophialora bantiana*.

Contributor's Morphologic Diagnoses: 1. Liver: Hepatitis, necrotizing and granulomatous, multifocal, severe.
2. Epiploon: Epiploitis necrotizing and granulomatous, diffuse severe.

Phaeohyphomycoses, etiology consistent with *Cladophialora bantiana*.

Contributor's Comment: The lesions in the liver were characterized by confluent foci of necrosis surrounded by histiocytic infiltration, including epithelioid cells and multinucleated giant cells, fewer neutrophils, lymphocytes, and plasma cells. A large number of pigmented fungal hyphae were seen in all areas of necrotic tissue and within the border of the lesions. The epiploon and all serosal surfaces of the abdomen (liver, spleen, intestine, abdominal part of the diaphragm) were covered by a layer of necrotic tissue containing a large number of pigmented fungal hyphae over a layer of predominantly macrophages with giant cells, epithelioid cells and some neutrophiles, lymphocytes, and plasma cells.

The term Phaeohyphomycosis is applied to a group of mycotic infections, subcutaneous or systemic, caused by fungi that grow in the host's tissues and have dark walled (dematiaceous) septate mycelia. The dematiaceous fungi capable of causing opportunistic infections are ubiquitous saprobes that are commonly associated with plants and soils. Most infections involve the dermis and subcutaneous tissues.

Systemic phaeohyphomycosis is rare. *Cladophialora bantiana* is the most commonly reported agent causing systemic phaeohyphomycosis in animals and humans. It is highly neurotropic. In humans, these infections are generally associated with drug-induced immune suppression.

In this case, the cat was serologically negative for FeLV, FIV and FIPV antigens. The cerebral localization was neither confirmed by macroscopic nor microscopic observation. The origin of the systemic manifestation of fungal infection and pathogenesis of the infection are still unknown.

AFIP Diagnoses: 1. Liver: Hepatitis, necrotizing, granulomatous, multifocal to coalescing, moderate, with numerous dematiaceous fungal hyphae, pseudohyphae, and yeast (phaeohyphomycosis), Domestic Longhair, feline.
2. Adipose tissue: Steatitis, necrotizing, granulomatous, multifocal to coalescing, moderate, with numerous dematiaceous fungal hyphae, pseudohyphae, and yeast (phaeohyphomycosis).

Conference Comment: Phaeohyphomycosis most commonly presents in a cutaneous form. This is typically the result of a penetrating foreign object resulting in traumatic implantation. A solitary, subcutaneous, firm or fluctuant mass develops at the site of inoculation. Dissemination is rare.

Systemic or cerebral infection is believed to occur following inhalation of fungal elements. Cerebral infection is most commonly a result of infection with *Cladosporium* sp., which has an unexplained neurotropism. The histologic lesion is reported as a suppurative to pyogranulomatous necrotizing meningoencephalitis.

Histological diagnosis of phaeohyphomycosis is based on the presence of branching, prominently septate hyphae with distinctive pigmentation; yeast may also be present. With sparsely pigmented fungi, special procedures such as the Fontana-Masson method may be helpful in identifying melanin pigment. Because the histomorphology between the various phaeohyphomycotic agents is similar, culture is necessary for a definitive diagnosis.

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CASE III – 393-97 (AFIP 2741017)

Signalment: 9-year-old, male Silvery Marmoset (*Callithrix argentata*)

History: Found dead in the exhibit. No ante-mortem signs were observed.

Gross Pathology: The peritoneal cavity contains multifocal to coalescing adherent flecks of yellow fibrinous material. The material is most abundant surrounding the urinary bladder, cecum and colon. The proximal portion of the cecum is thickened 4-5 times normal. On section, the thickened cecum is diffusely pale tan to white. A papillary carcinoma is present in the thyroid gland.

Laboratory Results: Cultures of the peritoneum isolated many and pure *Klebsiella pneumoniae*.

Contributor's Morphologic Diagnosis: Typhlocolitis, transmural, acute, necrotizing, severe with crypt herniation, diffuse edema, lymphangiectasia, necrotizing vasculitis and moderate to large numbers of rod bacteria.

Contributor's Comment: This animal was one of 10 individuals out of 26 silvery marmosets in the Wildlife Conservation Society (WCS) collection to die over a 7 month period of time. Of these, 7/10 died peracutely with severe typhlitis or typhlocolitis with ulceration and transmural abscessation with variable involvement of the cecal lymph nodes, serosal cavities, and other organs. Both males and females were affected. The ages ranged from 3 months to 9 years. *Klebsiella pneumoniae* was the predominant isolate from a variety of sites. Cultures were negative for bacteria commonly associated with intestinal disease in primates (*Campylobacter* sp., *Salmonella* sp., *Yersinia* sp., and *Shigella* sp.). In most cases, the marmosets died with no antemortem clinical signs. Two cases presented semi-comatose and subsequently died. One animal had a palpable mass in the caudal abdominal cavity, which corresponded to an abscessed cecal lymph node. Blood work was available on two animals. One animal had a regenerative leukocytosis with 29% band cells and both animals had pre-renal azotemia, hypergammaglobulinemia and anemia. Group treatments of surviving animals were attempted based on sensitivities from cultures taken at necropsy, however sporadic deaths continued during the seven-month time period.

This presentation was unusual in that the most common manifestation of *Klebsiella pneumoniae* in humans and other primates is pneumonia with subsequent

bacteremia and localization in distant sites. There is only one similar report of *Klebsiella pneumoniae* related enteric disease in a group of primates at a research facility in Iquitos, Peru. In the Peruvian outbreak, *Saguinus*, *Aotus*, and one *Saimiri* were affected. In the *Saguinus*, the primary lesion was purulent peritonitis with no intestinal involvement. In the *Aotus*, the most common lesion was typhlitis with lymph node abscessation and peritonitis. During the outbreak at WCS, the infection started in the cecum with local spread (colon, cecal lymph node and peritoneum) and subsequent bacteremia (intravascular bacteria) with spread to distant sites (pleural cavity, pericardium, and meninges). Bacteria were readily identified in smears and histopathology sections. In those cases where *Klebsiella pneumoniae* was isolated, bacteria identified in the lesions were Gram negative rods surrounded by a thick clear capsule, which supports the belief that *Klebsiella pneumoniae* was the cause of the disease. In all cases, even in cases with multiple aerobic and anaerobic bacterial isolates from the abdominal cavity, *Klebsiella pneumoniae* was isolated in pure culture from at least one site.

AFIP Diagnosis: Colon: Colitis, necrotizing, suppurative, acute, transmural, diffuse, severe, with hemorrhage, fibrin deposition, and myriad bacilli, Silvery marmoset (*Callithrix argentata*), nonhuman primate.

Conference Comment: *Klebsiella pneumoniae* is a non spore-forming member of the Enterobacteriaceae family and is often present in the intestine as a common commensal. An inhabitant of the environment, it is frequently found in soil and water. *K. pneumoniae* is generally not considered to be a primary pathogen, but an opportunist, and of the seven *Klebsiella* sp. only *K. pneumoniae* is pathogenic in animals.

K. pneumoniae is reported to cause cystitis in dogs, mastitis in cows, metritis and abortion in mares, and is a common cause of pneumonia in non-human primates. In primates, the pneumonia is typically characterized as a necrotizing fibrinosuppurative bronchopneumonia. Virulence is enhanced by the presence of fimbriae which act as adhesins, permit colonization of mucosal surfaces, and inhibit phagocytosis and intracellular killing. A heat stable capsule inhibits opsonization and phagocytosis.

The differential diagnosis discussed in conference included *Yersinia enterocolitica* (Gram negative), *Campylobacter jejuni* (Gram negative), *Salmonella* sp. (Gram negative), and *Shigella* sp. (Gram negative). Of the four *Shigella* sp. discussed, *S. dysenteriae*, which produces shiga toxin, is the most pathogenic; *S. flexneri* is the most common.

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CASE IV – 01-005-34/35 (AFIP 2787800)

Signalment: 16-year-old male, Rhesus macaque (Macaca mulatta).

History: Pancreatectomy donor on 10/4/00 (terminal surgery). He was euthanized with IV sodium pentobarbital. During the procedure there were anesthesia difficulties. The department of veterinary medicine requested evaluation of the lung.

Gross Pathology: This 8.72 kg 16-year-old male rhesus macaque was in a good nutritional state with moderate amounts of yellow subcutaneous and visceral fat stores. There are multifocal air-filled bullae or cysts in dorsal lung lobes. These cysts are of various sizes, have a yellowish wall and contain inspissated material and mucus. These multiple cystic lesions are often in the hilar region of lung lobes toward the dorsal aspects. There are no other abnormal gross findings.

Laboratory Results: None

Contributor's Morphologic Diagnosis: Lung, all lobes: Bronchopneumonia, eosinophilic and granulomatous, chronic, multifocal, marked, with bronchiolectasis, abundant anisotropic yellow-brown crystalline mite pigment, and adult mites, etiology consistent with *Pneumonyssus simicola*.

Contributor's Comment: *Pneumonyssus simicola*, the lung mite of macaques, is no longer a routine finding in colony-housed animals. This monkey may have been wild caught or infected prior to entry into the monkey colony.

Pulmonary acariasis occurs in up to 100% of wild rhesus monkeys. In both wild and colony-raised rhesus monkeys, *Pneumonyssus* infection is usually subclinical. Monkeys with severe infections may have coughing or dyspnea. Reported complications include pneumothorax and pulmonary arteritis.

Transmission requires close association with infected animals. Transmission can be prevented by separation of the mother and neonate shortly after birth. The details of the life cycle of the mite are unclear. The entire life cycle can apparently be completed within the host lung.

Gross lung mite lesions are focal, round, yellow to tan cystic foci up to several millimeters in diameter within the lung parenchyma. Mites occasionally can be visualized in the center of these lesions with the aid of a dissecting scope.

Histopathologic findings typically include granulomatous and eosinophilic inflammation centered on the terminal air passages, pigment-laden macrophages, bronchiectasis, alveolar emphysema, bronchiolar smooth muscle hyperplasia and interstitial fibrosis. The characteristic birefringent crystalline pigment is useful in making a presumptive diagnosis, even in the absence of adult mites. The pigment, regarded as a metabolite of female mites, causes most of the inflammatory reaction.

AFIP Diagnosis: Lung: Bronchiolitis and peribronchiolitis, granulomatous and eosinophilic, multifocal, moderate, with bronchiolectasis and adult mites, etiology consistent with *Pneumonyssus simicola*, Rhesus macaque (*Macaca mulatta*), non-human primate.

Conference Comment: *Pneumonyssus simicola* is a member of the family Halarachnidae. *P. simicola* are 300-500 um in diameter and have typical arthropod characteristics; a body cavity, striated musculature, jointed appendages, and a variably chitinized cuticle. In this case, highly chitinized points of muscular attachment are visible on the cuticle. Depending on section, the brain, gastrointestinal tract, reproductive structures, and yolk (brightly eosinophilic globular material) are also visible.

Numerous arthropods are reported to affect the respiratory tract in several species. Those discussed in conference include *Rhinophaga* sp. in the lungs of Old World monkeys, *Pneumonyssoides caninum* in the sinuses and nasal passages of dogs, *Cephenemyia* sp. in the nasal cavity and pharynx of wild cervids, *Cytodites nudus* in the air sacs of poultry, and *Halarachne* sp. in the nasal passages of sea lions.

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