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CASE I - 2422/02 (AFIP 2888767)

Signalment: Bennett's Wallaby (*Macropus rufogriseus*), 4 years, female, born and kept in zoo.

History: The animal showed increasing anorexia and a decline of body weight over a period of 2-3 months; prior to death it was lethargic/apathic. One week prior to death, the wallaby's approximately 15 cm large neonate was found dead within the cage. During the last years, several individuals of this group of Bennett's Wallabies suffered from lumpy jaw. Post mortem examination of this adult was performed one day after death; the neonate was not submitted for investigation.

Gross Pathology: The animal was emaciated (body weight: 9.5 kg). Bilaterally, the first and second premolars showed moderate tartar as well as a chronic purulent periodontitis, osteitis and ulcerative gingivitis (lumpy jaw). In the liver and spleen, multifocal to coalescing, well demarcated, whitish masses of 2 to 15 mm diameter were observed (Fig. 1). Few noncoalescing nodular whitish masses were found in femoral bone marrow and lactating mammary gland. The hepatic, splenic and cranial mesenteric lymph nodes were moderately enlarged and exhibited a homogeneous whitish cut surface. The lungs showed moderate alveolar edema and moderate acute congestion.

Laboratory Results: Microbiology: Liver, spleen, mammary gland: numerous acid-fast bacilli (*Mycobacterium avium* complex) (Fig. 2); liver, spleen, kidneys, lungs, small intestine, mammary gland: high amounts of *Escherichia coli* (+++).

Contributor's Morphologic Diagnosis: Liver, spleen, mammary gland, bone marrow: Hepatitis, splenitis, mastitis, osteomyelitis, pyogranulomatous and necrotizing, multifocal to coalescing, severe, chronic, with multifocal mineralisation and numerous intra- and

extracellular acid fast bacilli and gram-negative rods, partly intrasinusoidal and intravascular, Bennett's Wallaby (*Macropus rufogriseus*), marsupial.

Etiology: Infection with *Mycobacterium avium* complex and *Escherichia coli*

Contributor's Comment: Infections of marsupials kept in captivity with bacilli of the *Mycobacterium avium* complex (MAC; *M. avium, M. intracellulare*) are reported from North American Matchie's tree kangaroos (*Dendrolagus matschiei*). In this species, an increased susceptibility to opportunistic MAC infections with mainly pulmonary manifestation has been observed in relation to a reduced cellular immune reactivity, although additional factors, such as genetic influence, stress, and environmental exposure are not excludable[1]. In the present case, a similar type of MAC infection with manifestation in liver, spleen, mammary gland, bone marrow (not seen in all slides), but not in the lungs is presented in a Bennett's Wallaby. Acid-fast bacilli (MAC) and gram negative rods (*E. coli*) were additionally found in the cisternae of the teat of the lactating mammary gland, indicating a possible relation between the bacterial infection of the mother and the death of the suckling neonate.

The source of bacterial infection in this case could not be determined. Acid-fast bacilli of the MAC are of environmental origin. In immunosuppressed mammalian hosts, including humans, systemic diseases due to an infection with bacilli of *Mycobacterium avium* complex occur. MAC organisms persist and replicate within mononuclear phagocytes of the reticuloendothelial system[2]. They tolerate the acidic conditions of the stomach, resist the membrane-disrupting activity of cationic peptides, and invade intestinal epithelial cells[3]. Experimental studies on phagosomes containing *M. avium* showed that phagosomes are arrested in their maturation, lack lysosomal markers and are not acidified. Infected macrophages undergo apoptosis. Tumor necrosis factor-alpha (TNF-alpha) is one of the key cytokines elicited by macrophages infected with pathogenic mycobacteria. The TNF-alpha release of macrophages is regulated in a strain-, cell type-, and stimulus-specific manner. The *M. avium* induced production of TNF-alpha seems to be regulated by mitogen-activated protein kinases (MAPKs)[4, and references therein].

In contrast to the described cases in Matchie's tree kangaroos, the manifestation of mycobacteriosis in the Bennett's Wallaby was in the liver, the spleen, the mammary gland, the lymph nodes, and the bone marrow.

AFIP Diagnosis: Spleen, liver, bone marrow, and mammary gland: Granulomatous inflammation, diffuse, marked, with granulomas, Bennett's Wallaby (*Macropus rufogriseus*), marsupial.

Conference Comment: Marsupials are generally more susceptible to mycobacterial diseases than eutherian (true placental) mammals. The contributor summarized the incidence of MAC in Matschie's tree kangaroos. Other reports of mycobacterial

infection in marsupials include subcutaneous atypical mycobacteria in captive tiger quolls (*Dasyurus maculatus*)[6], cutaneous and respiratory infections by *Mycobacterium ulcerans* in koalas (*Phascolarctos cinereus*)[7], and *Mycobacterium bovis* infections in brushtail possums (*Trichosurus vulpecula*)[6]. Reduced cell-mediated immunity has been demonstrated in several marsupial species and may increase their susceptibility to mycobacterial infection. The cause of depressed cell-mediated immunity in these animals, however, is not known.[6]

This case demonstrates a striking amount of central necrosis in the granulomas, similar to that seen in cases of *Mycobacterium bovis* in white-tailed deer[8]. Conference attendees discussed the general pathology of granuloma formation. Interleukin-12 (IL-12) is produced by macrophages and induces T_H1 differentiation and interferon-gamma (IFN-gamma) secretion by T cells and natural killer (NK) cells. T_H1 cells secrete interleukin-2 (IL-2), IFN-gamma, and tumor necrosis factor (TNF). Interleukin-2 causes T cell proliferation. Interferon-gamma activates macrophages and causes further secretion of IL-12, enhances their ability to phagocytize microorganisms, and secretes polypeptide growth factors, such as platelet-derived growth factor and transforming growth factor-beta. These factors stimulate fibroblast proliferation and collagen synthesis, resulting in fibrosis if macrophage activation is sustained. Tumor necrosis factor-alpha exerts its effects on endothelium to facilitate the extravasation of lymphocytes and monocytes at the site of inflammation. All of these changes are characteristic of type IV hypersensitivity.[5]

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CASE II - 43305 (AFIP 2840998)

Signalment: Eight-year-old female red ruffed lemur (*Varecia variegata rubra*).

History: The lemur initially presented for evaluation of a large cervical mass after a 48-hr period of lethargy and anorexia. Physical exam revealed a soft fluctuant subcutaneous swelling extending from the dorsal aspect of the skull to the ventral cervical region. CBC and serum biochemistries were unremarkable. Radiographs of the cervical region showed discrete areas of mineralization in the mass. Ultrasonography of the mass was non-diagnostic. Surgical exploration of the area revealed a multiloculated mass with each cyst-like structure containing hundreds of <1mm bead-like nodules. Cestode larvae were identified upon examination of a wet-prep of the nodules. After a series of treatments with praziquantel, amoxicillin and albendazole, the mass was reduced to mild thickening of the ventral cervical region. No regrowth of the mass was detected during periodic examinations over the next year. Approximately one year post treatment, the cervical mass recurred. Despite surgical debulking and repeated anthelmintic treatment, the mass persisted and the lemur was finally euthanized.

Gross Pathology: The left ventrodorsal cervical region was moderately swollen from the caudal mandible to the thoracic inlet. Reflection of the skin revealed a gelatinous, multiloculated, infiltrative mass composed of thousands of soft round 1 to 2 mm coalescing cystic structures (cysticerci) joined by thin translucent membranous tissues. The mass encircled the trachea from the larynx into the thoracic inlet and dissected into adjacent subcutis, skeletal muscle and adventitia of the esophagus.

Laboratory Results: Microscopic examination of the cystic structures from the cervical mass revealed numerous cestode larvae. The morphology of the rostellar hooks and the characteristic exogenous budding identified the larvae as *Taenia crassiceps.*

Contributor's Morphologic Diagnosis: Subcutis and skeletal muscle: Severe regionally diffuse granulomatous and eosinophilic cellulitis and myositis with intralesional cysticerci (Etiology: *Taenia crassiceps*).

Contributor's Comment: *Taenia crassiceps* is a cestode parasite that is found commonly throughout North America, Europe and the former USSR and uses a variety of canids and occasionally felids as definitive hosts. Rodents are the most common reported intermediate hosts. Intermediate hosts become infected by ingesting oncospheres in the feces of a definitive host. Cysticerci develop in the subcutis and body cavities of the intermediate host and are subsequently consumed with body tissues by the predator definitive host. The extensive infections sometimes seen in intermediate hosts, such as this lemur, is a result of the ability of *T. crassiceps* to proliferate by budding both exogenously and endogenously. This ability also may explain the recurring and persistent nature of the infection. The enclosure in which this lemur was housed would not allow entry of any canid or felid but it is possible that a definitive host (most likely grey fox) defecated close enough to the enclosure so that the lemur could reach the feces. Cysticercosis has been reported in other non-rodent species, most recently in immunocompromised domestic dogs and humans.

AFIP Diagnosis: Skeletal muscle: Cysticerci, with granulomatous and eosinophilic myositis, red ruffed lemur (*Varecia variegata rubra*), nonhuman primate.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant for veterinary parasitology. Although measurement and description of hooks is necessary to definitively characterize the species, Dr. Gardiner believes this is *Taenia crassiceps* because this species has been found to asexually multiply in host tissue. Of interest, he notes the presence of degenerate cysticerci in the tissues surrounding the viable (at the time of fixation) cysticerci. Many times, only calcareous corpuscles and hooks are all that remain in tissue sections. An acid-fast stain may be used to highlight the hooks.

There are four basic types of second stage larval cestodes, which serve as the infective form for definitive hosts: cysticercus, strobilocercus, coenurus, and hydatid. A cysticercus consists of a single bladder with one scolex, whereas a coenurus consists of a single bladder with many scolices, each having the potential to develop into a mature tapeworm. A strobilocercus is a cysticercus that has already begun to elongate and segment while in the intermediate host.

Hydatids contain thousands of scolices (protoscolices) and are formed by members of the genus *Echinococcus*. Often protoscolices are grouped into small clusters termed brood capsules. When brood capsules rupture, the scolices are released to form a sediment called hydatid sand within the fluid-filled cyst cavity. Unilocular hydatid cysts are the second stage larvae of *Echinococcus granulosus*, for which dogs and wild carnivores serve as definitive hosts. Many species serve as intermediate hosts, including sheep, cattle, horses, swine, and humans. The dog-sheep cycle has been identified as one of the most important in many geographic areas. Hydatid cysts reside in the body cavities and viscera and may become extremely large, especially in human beings.[4,5,6]

Contributor: Zoological Society of San Diego, San Diego, CA

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CASE III - 211305 (AFIP 2910178)

Signalment: Adult, female, fish crow (*Corvus ossifragus*).

History: This fish crow was wild-caught in Maryland and used as a positive control in a West Nile virus (WNV) study*. It was humanely killed on day 10 post-infection.

*Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Gross Pathology: None reported.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Heart: Pancarditis, necrotizing, lymphoplasmacytic and histiocytic, multifocal to coalescing, severe.
2. Pulmonary artery: Arteritis, chronic, multifocal, moderate with adult filarial nematode, etiology consistent with *Splendidofilaria caperata*.

Contributor's Comment: This case is interesting because it has two lesions of different etiologies. The first lesion within the heart was caused by WNV and is characterized by multifocal to coalescing areas of myocardial degeneration and necrosis with infiltrates of lymphocytes, plasma cells, macrophages, and an occasional

heterophil. Similar inflammation was also observed multifocally within the endocardium and epicardium. The second lesion is a chronic pulmonary arteritis caused by a gravid, adult, female, filarial nematode embedded in the tunica media. *Splendidofilaria caperata* has been reported to inhabit the tunica media of the pulmonary artery in magpies and starlings in Colorado and crows in Ontario, Canada[4].

West Nile virus lesions are well characterized in birds[2]. In this study lymphoplasmacytic and histiocytic inflammation with necrosis often in a perivascular distribution was observed in just about every tissue in the body with the most severe lesions affecting the heart, skeletal muscle, and spleen. Meningoencephalitis was observed in every case but was mild. West Nile Virus infection of the brain in crows and magpies has been reported to be mild in comparison to WNV infection in many other bird species[2].

Other WNV lesions observed in this case included: hepatitis, splenic red pulp necrosis and lymphoid depletion, interstitial nephritis, ventriculitis (gizzard), dermatitis, myositis, coelomitis, and adrenalitis.

In the fall of 1999, WNV emerged for the first time in the western hemisphere in New York City during an outbreak of disease that involved humans, horses, and wild and exotic birds[1]. Since that time, the virus has rapidly spread to infect humans and animals in almost every state in the United States. West Nile Virus belongs to the family Flaviviridae in the Japanese encephalitis (JE) serocomplex group that includes Saint Louis encephalitis virus, Kunjin virus, and Murray Valley encephalitis virus[2]. WNV is somewhat unusual from the other flaviviruses in that it can be transmitted by several species of mosquitoes and ticks, orally through the consumption of infected birds and rodents[3], by direct contact[3], blood transfusion[5,7], organ transplantation[5,7], and intrauterine fetus infection[6]. There is even a strong suspicion of transmission to a human infant through breast milk[6]. West Nile Virus is also unusual in that it has a wide host range including many species of birds, mammals, and reptiles. The National Wildlife Health Center maintains a list of animal species found positive for WNV in surveillance efforts. As of September 4, 2003, over 200 avian species, 29 mammalian species and 2 reptile species have tested positive for the virus. (http://www.nwhc.usgs.gov/research/west_nile/wnvaffected_text.html).

The opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

AFIP Diagnoses:

1. Heart: Myocarditis, necrotizing, subacute, diffuse, severe, fish crow (*Corvus ossifragus*), avian.
2. Ganglion, heart base epicardial fat: Periganglioneuritis, lymphoplasmacytic, focal, mild.

3. Pulmonary artery: Endarteritis, proliferative, lymphoplasmacytic, diffuse, moderate, with intramural nematodes.

Conference Comment: Conference attendees noted that myocardial inflammation multifocally extends into the epicardium and endocardium. Lymphoplasmacytic inflammation also surrounds an epicardial ganglion, although an association with WNV infection is uncertain.

The genetic variant of WNV isolated in the United States is highly pathogenic in many species of birds, especially corvids. Other species of birds, such as chickens, are more resistant, although chickens do maintain a level of viremia adequate to infect mosquitoes and serve as an amplifier host. The reservoir host for WNV remains unknown.[1,3,8]

Differences in the histologic lesions and amounts of tissue viral antigen among several species affected by this virus have been documented. In crows and other wild birds, there is a large amount of viral antigen within both the CNS and extraneural organs, including the heart and kidney, whereas the amount of viral antigen detected in horses is minimal and limited to the CNS. Histologic lesions in free-ranging crows tend to be mild or absent, whereas lesions may be severe in other species of birds, such as strigiform owls, consisting of encephalitis and myocarditis.[10] Horses tend to exhibit moderate or severe histologic lesions, again, limited to the CNS. These consist of polioencephalomyelitis with perivascular infiltrates and hemorrhage. Horses may be considered incidental, dead-end hosts based on the presence of moderate to severe lesions, shortened viremia, and limited tissue distribution of viral antigen.[8,9,10]

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CASE IV - 1984-577 (AFIP 2835107)

Signalment: Female Parma (or white-fronted) wallaby (*Macropus parma*) of unknown age (likely an adult).

History: Not a collection animal. Neurologic signs for 1 week; unable to right herself; treated with SC fluids, dexamethasone, Flocillin, Tribrissen, vitamin E, ivermectin and Sulmet for coccidia; periods of improvement where she appeared almost normal; head tilt for 2 days prior to death.

Gross Pathology: This animal was in good flesh and had no gross lesions other than markedly thickened, pale green-tan friable esophageal mucosa.

Laboratory Results: Antemortem data unavailable. Immunohistochemistry on sections of brain is positive for *Toxoplasma gondii*.

Contributor's Morphologic Diagnosis: Brain: Meningoencephalitis, necrogranulomatous, chronic, multifocal, severe with disseminated protozoa consistent with *Toxoplasma gondii*.

Contributor's Comment: In all sections of brain examined there are multiple nodular infiltrates of lymphocytes, plasma cells and macrophages either associated or unassociated with foci of necrosis. In and around these lesions there are numerous approximately 15-60 um diameter protozoal cysts that are PAS and GMS positive. There are diffuse moderate meningeal and perivascular lymphoplasmacytic infiltrates.

Additional histologic findings include multifocal, mild to moderate, lymphoplasmacytic myocarditis, diffuse pulmonary edema and congestion and mild, random, multifocal lymphocytic hepatitis. Although these lesions are likely related to *Toxoplasma* infection, organisms were only seen in the brain. There was also a marked, diffuse, chronic bacterial esophagitis.

Toxoplasma gondii is a coccidian parasite with worldwide distribution. Virtually all vertebrate species are susceptible to infection.[1] Although infection may be common in many mammalian species, clinical disease is rare. Virulence of *T. gondii* strains, compromised immunity and species susceptibility may be factors in outbreaks of acute disease and death. Certain taxonomic groups and species of animals are highly susceptible to clinical and often fatal toxoplasmosis. These include Australian marsupials, New World monkeys, prosimians and slender-tailed meerkats.[1,3]

Clinical manifestations of toxoplasmosis vary with the species and organ system(s) affected. Signs may be localized as in ocular or CNS involvement or pneumonia or they may be generalized. As with other highly susceptible species, Australian marsupials often acquire overwhelming infection with *T. gondii* and may die peracutely without premonitory signs.[1,3,4,6]

Deaths due to toxoplasmosis have been reported in numerous species of Australian marsupials including wild and captive macropods (kangaroos, wallabies, wallaroos), captive koalas, wild bandicoots and possums and captive wombats and dasyurids. There are often no visible gross lesions at necropsy. In macropods, pulmonary congestion, edema and consolidation are commonly seen and may be the only significant necropsy findings.[2,4,5,6] Other macroscopic lesions reported in macropods include myocardial hemorrhages, often interspersed with pale streaks or foci,[3,5] cerebral malacia,[5] gastrointestinal ulceration[3,5] and splenomegaly and lymphadenomegaly.[4,5]

Microscopically, necrosis is the predominant lesion, especially in the CNS, lungs, lymph nodes, liver and muscle, with a variable inflammatory response.[1,3] Histologic lesions commonly seen in macropods include nonsuppurative meningoencephalitis +/- necrosis, glial nodules,[3-7] fibrinonecrotic pneumonia and/or pulmonary edema and congestion,[2-7] necrotizing lymphadenitis,[4,5,7] necrotizing hepatitis [4,5,7] and myocarditis and myocardial, skeletal and smooth muscle necrosis +/- mineralization.[3,5,7] Necroulcerative gastroenteritis, caused by tachyzoites released following ingestion of infective *T. gondii* tissue cysts or fecal oocysts, also occurs.[1,3,5,7]

Although the distribution of *T. gondii* organisms in tissues is variable, organisms are often widely disseminated. Intracellular aggregates of either bradyzoites or tachyzoites, extracellular tachyzoites and encysted organisms have been identified in various tissues.[5,7] Tissue cysts are especially common in brain and striated and smooth muscle and often are not associated with lesions.[4,5] Free zoites and intracellular aggregates of tachyzoites are often more common in extensive areas of necrosis.[5]

Diagnosis of the toxoplasmosis is based on demonstration of organisms in tissues by light and electron microscopy and *T. gondii* specific immunohistochemical staining, bioassay, tissue antigen ELISA or PCR analysis.[1] With light microscopy, *T. gondii* tachyzoites are 4-6 um long and oval to crescent shaped; cysts are 10-100 um, round to elongate, thin-walled (less than 0.5 mm thick) structures containing a few to several hundred slender, PAS-positive bradyzoites.[5,8] Antemortem diagnosis is aided by

serology. Detection of antibodies in serum suggest previous or current infection by *T. gondii*.[1,2] Non-species specific assays are available for use in exotic, non-felid animals. Paired IgG titers on serum taken 2-3 weeks apart and measured with the same test at the same time that show at least a fourfold rise in IgG are indicative of active infection.

Infection with *T. gondii* occurs via three possible routes of transmission. Carnivores are infected by ingestion of tissue cysts in raw muscle, liver or other tissues (raw meat diets or predation of infected mammals in birds). Transplacental infection is possible and was suspected in 3 wallaroos that died with toxoplasmosis.[6] Commonly, exposure of herbivores is through ingestion of hay or grain contaminated with feline feces containing infective sporulated oocysts.[1] In several reports of toxoplasmosis in captive macropods, which are herbivorous, there was evidence of domestic cat involvement or contamination of marsupial feed and/or exhibits by domestic cats was considered probable.[1,2,3,4,5,6,7] Activation of latent *T. gondii* infection by immunosuppression or stress (shipping, recent introduction, etc.) is also a possible source of organisms.[1,4] Reactivation of latent *T. gondii* infection in humans and mice is consistent with localized toxoplasmosis and the distribution of lesions in localized toxoplasmosis may reflect the common distribution of *T. gondii* tissue cysts in muscle and brain of latently infected hosts.[3]

The extreme susceptibility of certain groups and species of animals to toxoplasmosis may be explained by the following: arboreal habitat of New World monkeys and prosimians (no contact with food contaminated by cats), feeding ecology of herbivores/insectivores (absence or sporadic absence of meat in diet) and reduced evolutionary exposure to felids (prosimians and Australian marsupials).[1,3] There were no cats in Australia before settlement by Europeans, therefore it may be that marsupials were never exposed to *T. gondii* during the evolutionary process.[5]

AFIP Diagnosis: Brain: Meningoencephalitis, necrotizing, lymphoplasmacytic, multifocal, severe, with protozoal cysts, parma (white-fronted) wallaby (*Macropus parma*), marsupial.

Conference Comment: There is variation in the sections of brain provided by the contributor. This case was reviewed in consultation with Dr. J. P. Dubey, veterinary parasitology consultant to the Armed Forces Institute of Pathology.

The contributor gives an excellent review of toxoplasmosis. Conference attendees discussed *Neospora caninum* as a differential diagnosis for this lesion. Immunohistochemistry or electron microscopy is needed to differentiate these two organisms.

As noted by the contributor, Australian marsupials and New World monkeys are most susceptible to infection, whereas Old World monkeys, rats, cattle, and horses are highly resistant. Lesions vary among the different species affected. In small ruminants,

toxoplasmosis most commonly causes necrotizing cotyledonary placentitis, with characteristic 1-2mm diameter white foci of inflammation, necrosis, and mineralization. In dogs, pulmonary lesions may be severe, causing necrotizing interstitial pneumonia. Often in puppies, toxoplasmosis is triggered by immunosuppression caused by infection with canine distemper virus. In disseminated infection, other lesions include necrotizing hepatitis, myocarditis, splenitis, myositis, encephalitis, and ophthalmitis. In humans, toxoplasmosis is a common complication in immunosuppressed patients, such as those with AIDS or organ transplants. It can cause disseminated and often fatal parasitemia in the human fetus during the first trimester of pregnancy.[9,10]

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