C.L. Davis 2017
Northeastern Veterinary Pathology Conference

Case #7

CONTRIBUTORS/INSTITUTION:

Johns Hopkins University, School of Medicine
Department of Molecular and Comparative Pathobiology

SIGNALMENT:

7-month old, female, Blue duiker (*Philantomba monticola bicolor*) from the Maryland Zoo in Baltimore

HISTORY:

This duiker was transferred to the Maryland Zoo in Baltimore from another zoological institution in the fall of 2016. A pre-shipment screening exam was unremarkable, including physical exam, CBC and chemistry panel, TB testing, Brucella screening, and fecal screening. Previous vaccine history included West Nile Virus, Eastern Equine Encephalitis Virus, Western Equine Encephalitis Virus, Tetanus, CDT (*Clostridium perfringens* types C & D, and tetanus), and Rabies virus. After approximately 30 days in quarantine, the animal showed an abrupt and significant decrease in appetite. Physical exam was unremarkable at that time. Over the next several days, clinical signs progressed to include lethargy, ataxia, periocular hyperemia, pyrexia (105 F), and seizures. Serial CBCs were unremarkable, although blood smears showed hemophagocytosis, increased numbers of Howell-Jolly bodies, and unusually large white blood cells. Changes in serial chemistry screening was limited to mild to moderately increased liver enzymes (LDH, AST, and ALT). Treatments during this time included fluid therapy, enrofloxacin, oxytetracycline, and meloxicam. Minimal to no clinical response was noted. The animal was found dead in the morning on 12/3/16. At the previous institution, this animal had a brief episode of polyuria with possible stranguria and hematuria. Urinary tract infection was suspected, and signs resolved following treatment with oral antibiotics (TMS).

GROSS FINDINGS:

Bilaterally, the anterior chamber of the eye contained a small amount of freely movable, tan material (hypopyon). The subcutaneous tissue medial to the right stifle joint and extending caudally halfway up the femur was dark red (hemorrhage). The caudal thigh muscles showed extensive areas of brown to grey discoloration with multifocal areas of dark red to black (hemorrhage and necrosis). The right popliteal lymph node measured 2x2x1.5 cm and bulged on cut section. The contralateral limb and popliteal lymph node were grossly unremarkable. Multifocal, pinpoint to 3 mm, raised, white foci were on the surface of the kidneys bilaterally that also extended into the cortical parenchyma. The perirenal lymph nodes were prominent. The following lymph nodes were also enlarged: tracheobronchial and mesenteric. The brain and spinal cord were grossly unremarkable.

HISTOPATHOLOGIC/CYTOLOGIC FINDINGS:

Cerebrum (level of basal nuclei). Primarily affecting grey matter of the cortex and basal ganglia are multifocal, mild to moderate perivascular infiltrates composed of predominantly small and large lymphocytes and macrophages. The inflammatory cells occasionally extend into the adjacent neuropil where they are mixed with low numbers of reactive glial cells (reactive gliosis). There is also mild vascular congestion. The meninges are expanded by multifocal to coalescing areas of perivascular infiltrates of similar mononuclear inflammatory cells and rare multinucleated giant cells that contain nuclei arranged around the periphery of the cell (Langhans-type giant cells). In some areas, the meningeal inflammation extends into the underlying neuropil.

Kidney. Affecting approximately 25% of the renal cortex are multifocal to coalescing areas of interstitial and perivascular inflammatory infiltrates that frequently separate and replace renal tubules. Inflammatory cells are composed of predominantly lymphocytes and macrophages. There are also occasional multi-nucleated giant cells that contain up to 12 nuclei arranged around the periphery of the cell (Langhans-type giant cells). Similar inflammatory cells are present in the interstitium surrounding the renal pelvis. There is also mild to moderate vascular congestion

MORPHOLOGIC/ETIOLOGIC DIAGNOSIS:

Brain (cerebrum), meningoencephalitis, lymphohistiocytic, multifocal and perivascular, moderate, with reactive gliosis

Kidney, nephritis, lymphohistiocytic, multifocal and perivascular, severe

DISCUSSION:

The underlying cause of death in this duiker was severe, widespread inflammation affecting nearly every organ system including the brain and spinal cord, which likely resulted in the animal's clinical neurologic signs (seizures, ataxia). Moreover, in lymphoid tissue (spleen, thymus, lymph nodes), there was generalized loss of normal mature lymphocyte populations with replacement by similar inflammation and moderate to marked hemophagocyotosis. The character of the inflammation in this case was unusual, as infiltrates were typically perivascular and composed primarily of pleiomorphic mononuclear cells with large, irregular nuclei and prominent nucleoli (suggestive of either lymphoblasts or macrophages), fewer mature lymphocytes, and variable numbers of multinucleated giant cells. Immunohistochemistry was performed to further characterize the inflammatory cells. Multinucleated cells stained strongly with the macrophage marker Iba1, while the mononuclear cells were composed of a mixture of Iba1-positive cells and CD3 positive T lymphocytes. CD20 immunostaining was also performed to identify B cells, but the primary antibody did not cross-react with the duiker tissues.

Histochemical stains for bacteria, mycobacteria, and fungi were negative in all sections examined. Given the perivascular nature of the inflammation and clinical signs, as well as lack of other etiologies, a viral cause was initially suspected. PCR testing was performed for the following viruses: Rabies, Malignant Catarrhal Fever, Bluetongue, Epizootic Hemorrhagic Fever, Eastern Equine Encephalitis Virus, and Western Equine Encephalitis Virus. The results were negative for all diseases tested. Virus isolation for Bovine Adenovirus was also negative.

Overall, the histologic findings and lack of detectable etiologic agent in this case suggest an immune-mediated process. While little is known about immune-mediated diseases affecting exotic animal species, the lesions in this duiker did bear some similarity to systemic histiocytosis

(SH), a generalized proliferative histiocytic disease reported in young to middle-aged dogs. Similar to cutaneous histiocytosis, SH is a disease of interstitial dentritic cells, which are typically found in perivascular areas in multiple organs, with the exception of the brain neuropil. The inflammatory component of SH includes predominantly lymphohistiocytic inflammation that is vasocentric and often obliterates surrounding tissue and invades vessel walls (vasculitis). While the skin is often a site of inflammation, multiple other organs can be affected with SH. In this duiker, the inflammatory infiltrates were similar to SH in morphology and location, though overt vasculitis was not a prominent feature. Moreover, multinucleated giant cells, which were very common in this case, are rarely seen with SH. Additionally, the inflammation noted in the skin did not have the typical “bottom heavy” appearance that is usually seen with systemic histiocytosis.

This case also shared similar features with a rare condition in human medicine known as hemophagocytic lymphohistiocytosis (HLH). HLH is most frequently reported in infants up to 18 months of age, but is also observed in children and adults. This syndrome is associated with excessive inflammation leading to adjacent tissue damage, and is thought to be caused by dysregulation of activated macrophages and lymphocytes with a subsequent cytokine storm and organ failure. This disease can occur as a primary condition related to one of several known familial mutations, or secondary to a range of immune triggers such as viral and bacterial infections, lymphoma, and other autoimmune disease. Similar to human cases of HLH, this duiker had severe, multi-organ lymphohistiocytic inflammation with prominent erythrophagocytosis. Furthermore, the initial clinical presentation of fever and neurologic signs are consistent. However, patients with HLH also have anemia and thrombocytopenia as well as elevated liver enzymes. The cytopenias were not present in our case, as CBCs were within normal limits. Moreover, although the liver enzymes in this animal were mild to moderately elevated, an increase in LDH and AST could have been due to the muscle hemorrhage/necrosis noted in the gross examination. Thus, it is unclear if these elevated enzymes are due to the primary systemic inflammation or other factors. Finally, potential immune triggers in this duiker included the bacterial urinary tract infection reported by the previous institution, as well as a delayed-type hypersensitivity reaction to a vaccine or vaccine component.

This case is an unusual presentation of severe, systemic inflammation of uncertain etiopathogenesis in a young animal. Although a definitive diagnosis was not achieved, a proliferative, immune-mediated process is highly suspected.

REFERENCES:

1. Callan RJ and Van Metre DC. (2004). Viral diseases of the ruminant nervous system. Vet Clin Food Anim. 20: 327-362.
2. Moore PF. (2014). A review of histiocytic disease of dogs and cats. Vet Pathol. 51(1): 167-184.
3. Filipovich, AH and Chandrakasan S. (2015). Pathogenesis of hemophagocytic lymphohistiocytosis. Hematol Oncol Clin N Am. 29: 895-902.
4. Allen CE and McClain KL. (2015). Pathophysiology and Epidemiology of hemophagocytic lymphohistiocytosis. Hematology. 1:177-182.