Case 17

SIGNALMENT: Two-year-old intact male German shepherd dog (*Canis familiaris*) Military Working Dog (MWD)

HISTORY: Newly acquired MWD from Lackland Air Force Base, San Antonio, TX. History of intermittent diarrhea treated successfully with Metronidazole. No other significant prior medical history. The dog presented on emergency with severe ascites and lethargy. The dog went into cardiac arrest during exploratory laparotomy

GROSS FINDINGS: Heart: Multiple small yellow-white myocardial streaks. Severely dilated right ventricle

Abdominal cavity: Five liters of serosanguineous fluid in the abdomen.

Liver: Hepatomegaly with caudate liver lobe most affected by blunted and rounded edges.

HISTOPATHOLOGIC/CYTOLOGIC FINDINGS: Heart: Multifocally, cardiac myocytes are separated, surrounded, and replaced by high numbers of lymphocytes, plasma cells, and macrophages, with fewer neutrophils and fibroblasts with multifocal areas that contain eosinophilic beaded to finely fibrillar material (fibrin), and edema with increased clear space and ectatic lymphatics (edema). Up to 30 percent of remaining cardiac myocytes are degenerate with swollen, pale and vacuolated sarcoplasm, or necrotic with shrunken, angular hypereosinophilic or fragmented sarcoplasm with pyknosis or karyolysis and loss of cross striations.

Liver: Diffusely throughout this section of liver there are dilated and congested sinusoids in the centrilobular, midzonal and periportal areas. The central veins are also dilated and congested with mild thickening of the endothelium by fibrous connective tissue. The hepatocytes in the centrilobular and midzonal areas are compressed and atrophic or shrunken with hypereosinophilic cytoplasm and pyknotic nuclei (necrosis). Extending from the midzonal to periportal areas hepatocytes are swollen with pale vacuolated cytoplasm with and vesiculate nuclei (vacuolar degeneration). Scattered throughout the section, there are numerous hemosiderin laden Kupfer cells, and some hepatocytes contain a yellow/green granular pigment centered around bile ducts (bile worms, cholestasis). In the portal areas multifocally lymphatic vessels are widely dilated and contain a pale homogenous eosinophilic fluid (edema). There are prominent biliary profiles with increased number of ectatic bile ducts (biliary hyperplasia). The capsule is diffusely undulant with mild capsular fibrosis.

Abdominal fluid: Transudate (serosanguineous – 4% PCV)TP = 3.2 g/dL; Cellularity=no nucleated cells

MORPHOLOGIC/ETIOLOGIC DIAGNOSIS:
Heart: Myocarditis, lymphoplasmacytic and histiocytic, multifocal moderate with myocardial degeneration, necrosis, and fibrosis.
Liver: Liver: Hepatocellular cord atrophy, centrilobular, diffuse, marked, with sinusoidal congestion, and lymphatic dilation and edema (chronic passive congestion).

Etiologic Dx: Suspect trypanosomal myocarditis (Chagas disases)

DISCUSSION: *Trypanosoma cruzi* is the etiology of American trypanosomiasis, also known as Chagas disease, which can cause a fatal chronic myocarditis in both dogs and humans in endemic areas.[1-8] *T. cruzi* is a hemoflagellate protozoan of the family *Trypanosomatidae* and is endemic in South and Central America, Mexico, and in the southern United States (Texas, Arizona, New Medico, California, and Oklahoma).Dogs serve as the main reservoir for human infection;[1,3,6,8] however, the protozoa is maintained in wildlife reservoirs, including opossums, armadillos, rodents, cats, cats, raccoons, and monkeys. The protozoa are transmitted by a reduviid arthropod vector of the genus *Triatoma,* also known as "kissing" or "assassin" bugs.[1,4,6,8] These insects are primarily nocturnal and feed on the blood of mammals, birds, and reptiles. They have five nymphal stages and all stages can carry and transmit *T. cruzi*. The probability a triatomine is infected with T. cruzi increases with the number of blood meals taken. Therefore, older juvenile stages and adult arthropods have the highest rates of infection. *T.cruzi* is shed in the feces of the reduviid bug and transmitted to the mammalian host via excoriation of the site where the vector bit the host.[4,6,7,8]

T. cruzi has four distinct morphologic forms. These include the infective extracellular flagellated trypomastigote form. This form is only found in the blood and can be readily identified in blood smears of acutely infected mammals.[4] The intracellular amastigote form lacks flagella and multiplies via binary fission within mammalian cells, primarily cardiac myocytes. This stage is identified within intracellular tissue cysts. Dividing and proliferating amastigotes can lyse the cell and transform into flagellated trypomastigotes to auto-infect new sites within the body or enter the bloodstream and be ingested by the vector to complete the life cycle.[4] The epimastigote and promastigote forms are only found in the vectors and will not be present within infected mammalian hosts.[4,6]

In this case, amastigotes within myocardial cysts are not identified. In chronically infected animals, there is a scarcity of the protozoa and amastigotes are commonly not found, despite serial sectioning.[3,5] Serologic testing for specific antibodies to *T. cruzi* is the cornerstone of diagnosing chronic *T. cruzi* infection in both dogs and humans.[3,4,6] In this case, banked serum ELISA from January 2015 was negative. Banked serum ELISA from August 2015, just one month prior to death, was positive at 1:6384. Positive titers indicate exposure and are not definitive for infection. PCR on formalin fixed tissue from the right ventricle was negative for *T. cruzi*, but PCR assays are not useful in chronically infected animals due to the previously mentioned low parasite burden.[6] Differential diagnoses for lymphoplasmacytic and histiocytic myocarditis in dogs include a number of viral, bacterial, fungal, and parasitic causes.[4] However, given this dog's history of recently arriving from endemic San Antonio, Texas, a recent positive serum titer, and presence of the characteristic mononuclear myocardiitis, we highly suspect Chagas disease as the cause of death in this MWD. Widespread myocarditis likely resulted in a fatal arrhythmia leading to heart failure. Heart failure, in this case, is also supported by moderate to severe chronic passive congestion in the liver and resulting ascites noted in the history.

In dogs chronically infected with *T. cruzi*, myocarditis and myocardial degeneration/necrosis is often unassociated with the parasite, as seen in this case. The pathogenesis of this lesion pattern

is not completely understood, but there are likely multiple mechanisms occurring simultaneously. These include direct damage to myocardial cells by the parasite, parasite-specific immunity, bystandar cell damage, microvasculopathy, and autoimmunity to cardiac myocytes due to breakdown in self-tolerance and/or molecular mimicry.[2,3,4]

REFERENCES:

1. Bern C, Kjos S, Yabsley MJ, Montgomery SP. *Trypanosoma cruzi* and Chagas' disease in the United States. *Clin Microbiol Rev.* 2011; 24: 655-681.
2. Bilbe G. Overcoming neglect of kinetoplastid diseases. *Science*. 2015; 348(6238):974-976.
3. Bonney KM, Engman DM. Autoimmune pathogenesis of Chagas heart disease: Looking back, looking ahead. *Am J Pathol*. 2015; 185:1537-1547.
4. Center for Disease Control. Parasites - American Trypanosomiasis (also known as Chagas Disease) http://www.cdc.gov/parasites/chagas/epi.html.
5. Esper L, et al. Role of SOCS2 in modulating heart damage and function in a murine model of acute Chagas disease. *Am J Pathol*. 2012; 181(1):130-40.
6. Greene CE. African trypanosomiasis. In: Greene CE, ed. *Infectious Diseases of Cats and Dogs*. 4th ed. St. Louis, MO: Elsevier-Saunders; 2012:730-34.
7. Kapelusznik L, *et al.* Chagas disease in Latin American immigrants with dilated cardiomyopathy in New York City. *Clin Infect Dis.* 2013 Apr 12 [Epub ahead of print].
8. Nagajyothi F, Kuliawat R, Kusminski CM, Machado FS, Desruisseaux MS, Zhao D, Schwartz GJ, Huang H, Albanese C, Lisanti MP, Singh R, Li F, Weiss LM, Factor SM, Pessin JE, Scherer PE, Tanowitz HB. Alterations in glucose homeostasis in a murine model of Chagas disease. *Am J Pathol*. 2013; 182(3):886-94.
9. Woodhall D, et al. Neglected parasitic infections: What every family physician needs to know. *Am Fam Physician.* 2014; 89(10):803-11.