Diagnostic Exercise
From The Davis-Thompson Foundation*

Case #: **163** Month: **April** Year: **2021**

Title: Systemic Toxoplasmosis in a Red Kangaroo (*Macropus rufus*)

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Clinical History: A 1.5-year-old, intact male red kangaroo (*Macropus rufus*) was found dead in its pen and submitted for postmortem examination to the Louisiana Animal Disease Diagnostic Laboratory (LADDL).

Necropsy Findings: The thoracic cavity was filled with small amount (approximately 50 ml) of red-tinged fluid. Bilaterally, approximately 90% of the pulmonary parenchyma was dark red to purple and firm and failed to collapse, with some rib impressions on the dorsal surface. The pericardial sac contained approximately 10 ml of red-tinged fluid and the epicardium along the coronary groove had multifocal, short, white streaks.

Gross Images:

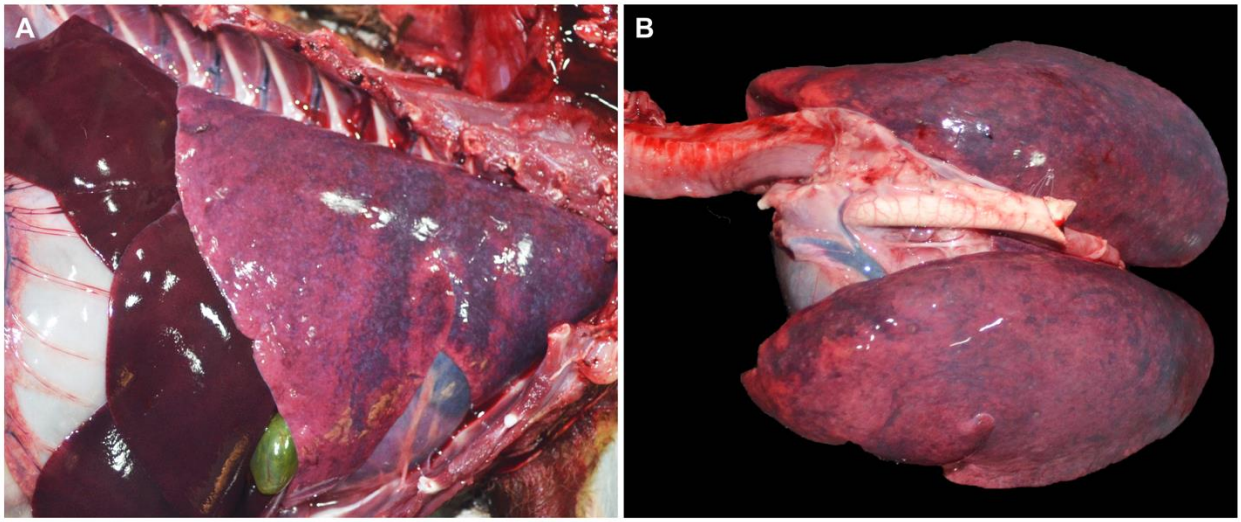

Figure 1: (A) Lateral view of the open thoracic cavity. (B) Lungs, dorsal view after being removed from the thorax.

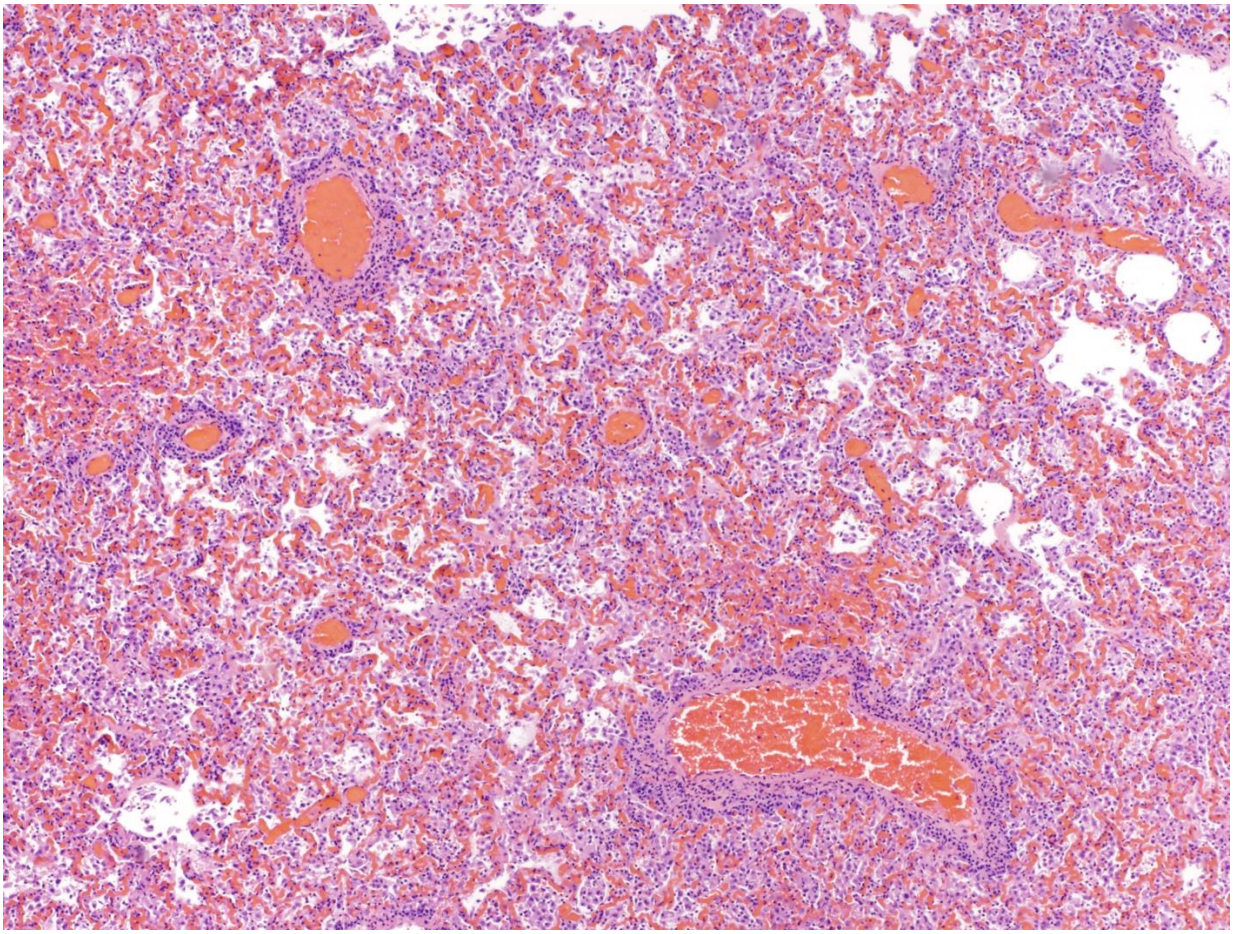

Figure 3: Lung. Alveolar spaces are diffusely filled by abundant histiocytes and alveolar edema. Pulmonary vessels and airways are delimited by cuffs composed of moderate numbers of lymphocytes, histiocytes and fewer plasma cells. H&E, 50X.

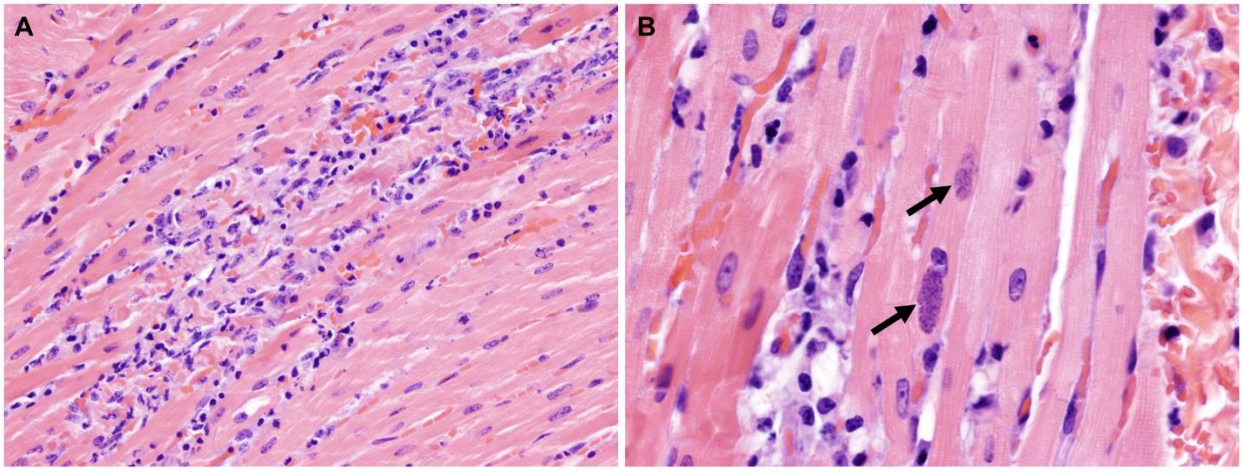

Figure 4: (A) Myocardium. Multifocal areas of the myocardium have fragmented cardiomyocytes, karyorrhectic debris, and mixed inflammatory cells including histiocytes, lymphocytes and plasma cells. H&E, 200X. (B) Myocardium. Occasionally within affected areas, cardiomyocytes contain intrasarcoplasmatic protozoal cysts (arrows). H&E, 400X.

- **Histologic description**

Lungs: The pulmonary parenchyma is diffusely and markedly congested, with alveolar spaces diffusely filled by abundant histiocytes and edema fluid. The pulmonary interstitium, most predominantly that around pulmonary vessels and occasional airways, is diffusely expanded by moderate numbers of lymphocytes, histiocytes and fewer plasma cells.
Heart: Multifocal areas throughout the myocardium are affected by necrosis characterized by foci of fragmented myofibers with increased sarcoplasmic eosinophilia and replacement by few histiocytes and lymphocytes, karyorrhectic debris, and proliferating fibroblasts. Occasional affected areas contain rare intrasarcoplasmatic protozoal cysts measuring approximately 15 x 5 µm and filled with numerous basophilic zoites.

• Morphologic diagnoses
Lung: Pneumonia, interstitial, histiocytic, lymphocytic and plasmacytic, diffuse, moderate, subacute, with marked alveolar histiocytosis.
Heart: Myocarditis, necrotizing, multifocal, moderate, subacute, with intralesional protozoal cysts.

• Associated lesions/other organs affected
Necrotizing myositis (skeletal muscle), necrotizing hepatitis, necrotizing encephalitis, lymphohistiocytic to necrotizing chorioretinitis and optic neuritis.

•Possible causes
Toxoplasma gondii
Neospora caninum
Trypanosoma cruzi (depending on the geographic location)

Discussion:
Toxoplasma gondii is a zoonotic apicomplexan protozoal organism closely related to *Neospora caninum* and *Sarcocystis* spp., all of which are known to cause disease in numerous mammalian species. While all of these have similar life cycles, they involve different definitive hosts. For *T. gondii*, the definitive hosts are cats and other wild felids, in which the parasite completes an intestinal cycle leading to fecal shedding of infective oocysts into the environment. A diverse number of domestic and wild mammals can act as intermediary hosts for *T. gondii* (including but not limited to carnivores, ruminants, cetaceans, fishes, rodents, primates, and marsupials), which acquire the infection by ingestion of sporulated oocysts from the environment and can either remain subclinical or develop severe disease. The disease pattern is mostly associated with individual susceptibility as well as other host factors such as the immune response. Rapidly dividing tachyzoites disseminate systemically within the intermediate host, gradually undergoing encystation within tissues and switching to a slowly dividing cell phenotype (bradyzoites). Macropodids, including kangaroos, are known to be intermediate hosts highly susceptible to develop severe and systemic disease, like the case presented here. Infections have been reported in red (*Macropus rufus*), eastern grey (*Macropus giganteus*) and western grey kangaroos (*Macropus fuliginosus*). While a wide range of tissues can be affected, the muscular and nervous tissues are common sites were tissue cysts containing bradyzoites develop. Histologic and immunohistochemical differentiation of protozoa from the phylum Apicomplexa is

challenging due to the lack of distinct histomorphological features and extensive cross-reactivity among antibodies available for immunohistochemistry and immunofluorescence. Consequently, molecular methods are often necessary to reach a definitive diagnosis. The infection in kangaroos (as well as other intermediate hosts including humans) is acquired from the environment (food, water sources or soil) contaminated with infective cat feces. Therefore, the only means to control this disease in marsupial populations is to avoid contamination of the food/water supplies and environment by limiting access by outdoor cats or other wild felids into the premises. In humans, consumption of raw and undercooked meat as well as unpasteurized goat milk are important additional transmission routes of *T. gondii* and, consequently, mitigation strategies also focus on measures to guarantee food safety (proper cooking of meat products and consumption of pasteurized milk-derived products).

Trypanosoma cruzi (the agent associated with Chagas disease) is an important rule-out depending on the geographical location. This zoonotic protozoal organism is a non-apicomplexan, and its transmission cycle involves an insect vector (triatomine bug). Despite limited occurrence within the US, cases of *T. cruzi* infection are sporadically seen in companion animals (dogs), particularly in the Southern US. Histologically, a distinctive feature of *T. cruzi* amastigotes, which are preferentially found in the cardiac and skeletal muscles, is the presence of a kinetoplast adjacent to and parallel to its nucleus.

Recommended Literature:

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