Title: Nestling male great horned owl (*Bubo virginianus*); body as a whole: leukocytozoonosis

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**Diagnoses:** Blood smear: Leukocytosis, with intraleukocytic protozoa, anemia and thrombocytopenia; body as a whole: Marked, chronic, leukocytozoonoses with leukocytosis; liver: Acute random, multifocal necrosis.

**Microscopic Findings:** Numerous protozoal megaloschizonts are present in a variety of tissues, including liver, spleen, lungs, myocardium, kidneys, and bone marrow, with highest concentrations in the liver, kidneys, and marrow spaces of scleral ossicles. Megaloschizonts are intracellular, variably shaped, and range from 50–140 μm in diameter. They are often subdivided into 3–6 discrete packets, and are replete with 1–2-μm-long, basophilic merozoites. Affected host cells often have markedly enlarged nuclei, up to approximately 40 μm in diameter. Rarely, small numbers of heterophils, lymphocytes, and macrophages form thin, partial cuffs surrounding schizonts; most often there is no associated inflammation. Blood vessels and hepatic sinusoids are often diffusely, moderately expanded by a mixed but primarily mononuclear inflammatory cell population, and smaller numbers of mature erythrocytes. Circulating mononuclear inflammatory cells occasionally have indented, peripheralized nuclei (suspect intracellular protozoa). In sections of the liver, there are multifocal, randomly distributed foci of coagulative necrosis characterized by loss of cytoplasmic detail and nuclear differential staining, bordered by peripheral zones of moderate hemorrhage.
Ancillary diagnostics: Liver and spleen tissues were submitted for PCR identification of protozoal parasites. DNA was extracted, and amplification of the cytochrome-b (cyt-b) gene yielded a 532-bp sequence, with 99% sequence identity to *Leucocytozoon* spp. PCR for *Haemoproteus* spp. was negative.
Discussion: The diagnosis of leucocytozoonosis was made based on morphologic features of parasite stages in erythrocytes, leukocytes, and endothelial cells, and confirmed by PCR. Leucocytozoon species comprise a broad genus of hemoparasitic apicomplexan protozoan parasites that infect a variety of bird species. The lifecycle is indirect, with infectious sporozoites transmitted to avian hosts by black fly (Simulium spp.) vectors. All leucocytozoon species are transmitted by Culicoides spp. midges. Sporozoites undergo merogony in hepatocytes,
forming schizonts. Individual merozoites released from schizonts can infect erythrocytes and leukocytes, forming intracellular gametocytes that characteristically distort the host cell and peripheralize the host nucleus. Immature gametocytes, as observed in the blood smear from this patient, may be more pleomorphic and difficult to distinguish from other hemoparasites. Syncytial merozoites can also be released from schizonts, and these can infect phagocytic cells to form megaloschizonts, ultimately releasing thousands of merozoites into the blood stream.1 Gametocytes in blood are infectious to insect vectors, and the cycle propagates with subsequent blood-meals.

Most Leucocytozoon infections are asymptomatic, and are encountered as incidental findings in blood smear analysis or postmortem examination. In young, debilitated, or otherwise immunocompromised animals, leucocytozoonosis can be a cause of clinically significant disease.2,3 This typically manifests as a profound anemia, likely secondary to an “anti-1 erythrocytic factor” produced by the meronts or by the host cell in response to infection. Leukocytosis is also a common feature of pathogenic leucocytozoonosis. Megaloschizonts can cause tissue damage from occlusion of vessels (as was suspected in the liver in this case), and rupture of megaloschizonts can be associated with tissue damage and inflammation. In Strigiformes, leucocytozoonosis is caused by *Leucocytozoon danilewskyi (Leucocytozoon ziemann)*. Several clades are recognized within this species, with variable host specificity between clades.
References:


*The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation. These exercises are contributed by members and non-members from any country of residence. Consider submitting an exercise! A final document containing this material with answers and a brief discussion will be posted on the CL Davis website (http://www.cldavis.org/diagnostic_exercises.html).

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