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Original Article

Case Series: Virulent hemosporidiosis infections in juvenile great horned owls (*Bubo virginianus*) from Louisiana and California, USA

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ABSTRACT

A total of eight juvenile great horned owls (*Bubo virginianus*) were found lethargic and on the ground in spring 2015, 2016, and 2017, including one fledgling from Louisiana, USA and seven nestlings from California, USA. One bird survived to release after rehabilitation; seven birds died or were euthanized due to poor prognosis and were necropsied. Necropsy findings were similar and included general pallor of all tissues, particularly the subcutis and lungs, and enlarged liver and spleen. Histopathology revealed multi-organ necrosis, abundant meronts containing merozoites, and intracytoplasmic pigmented haemosporidian parasites in blood cells in one bird. *Leucocytozoon* lineages lSTOCC16 and BUVIR06 were identified by polymerase chain reaction and genetic sequencing. The systemic *Leucocytozoon* infections were likely associated with morbidity and mortality in these owls. A second parasite, *Haemoproteus* lineage hSTVAR01, was also identified in an owl from Louisiana. This is the first identification of *Leucocytozoon* lineages that have been associated with mortality in young great horned owls.

1. Introduction

Infections with protozoan parasites in the order Haemosporida (genera *Haemoproteus*, *Plasmodium*, or *Leucocytozoon*) are common in many bird species, including raptors (Atkinson, 2008). Parasites within these genera differ in many aspects, including pathogenicity, global and regional distributions, mechanisms of parasite reproduction, and the vectors involved in transmission (Valkiūnas, 2005). *Haemoproteus* (*Paraphaemoproteus*) spp. and *Haemoproteus* (*Haemoproteus*) spp. are transmitted by biting midges (Ceratopogonidae) and louse flies (Hippoboscidae), respectively, and *Plasmodium* spp. are transmitted by mosquitoes (Culicidae). There are two known groups of vectors for *Leucocytozoon* spp.; *Leucocytozoon caulleryi* (although some consider this parasite to be in the subgenus Akiba) is transmitted by biting midges (Ceratopogonidae) while the remaining *Leucocytozoon* spp. are transmitted by black flies (Simuliidae) (Atkinson, 2008; Forrester and Greiner, 2008). Avian malaria is a significant conservation issue for some avian species (such as the native avifauna of Hawaii or penguins in zoological parks), but mortality in natural hosts occurs and is often likely underreported (Beier et al., 1981; Dinhopl et al., 2015; Valkiūnas and Iezhova, 2017). Nevertheless, potential subclinical effects on reproductive success and the short- and long-term survivability of natural hosts have been investigated in many bird species naturally infected with haemosporidian parasites (Appleby et al., 1999; Korpimaki et al., 1993; la Puente et al., 2010; Merino et al., 2000; Nordling et al., 1998; Remple, 2004; Ziman et al., 2004).

The severity of clinical disease in avian hosts depends on many factors, including the species of the host, the host's immunity, environmental stressors, and the species of haemosporidian involved, among others (Atkinson, 2008). Clinical disease can be broadly

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classified as causing one of two overlapping processes: (1) anemia due to erythrocyte rupture from developing gametocytes and (2) visceral organ inflammation, necrosis, and hemorrhage following the rupture of meronts (schizonts) and megalomeronts. Fatal infections in many bird species have involved varying degrees of inflammation in visceral organs and necrosis of cardiac and skeletal muscle, liver, lung, and kidneys associated with developing parasites (Alley et al., 2008; Beier et al., 1981; Donovan et al., 2008; Julian and Galt, 1980). Many of these cases involve captive exotic birds infected with haemosporidians not normally found in their native range, and in such cases parasites may not always be found in the blood but tissue stages may be extensive (termed 'abortive infections' because infective gametocytes are not produced) (Valkiūnas and Iezhova, 2017). However, there are also examples of disease developing in native hosts infected with native parasites and include wild turkeys (*Meleagris gallopavo*) and Canada geese (*Branta canadensis*) (Atkinson and Forrester, 1987; Herman et al., 1975).

In North America, haemosporidian infections are relatively common in owls (Gutierrez, 1989; Ishak et al., 2008). Historically, the only morphologically distinct species of *Haemoproteus* reported to cause disease in owls were *H. noctuae* and *H. syrnii* (Evans and Otter, 1998; Forrester et al., 1994; Valkiūnas, 2005). These morphospecies are thought to have near cosmopolitan distributions in birds of the order Strigiformes (Valkiūnas, 2005). To date, only two studies have reported *Haemoproteus* infections in great horned owls including in a single bird in Florida and in 11% of 54 birds from California (Ishak et al., 2008; Outlaw and Ricklefs, 2009). In both studies, infection was determined by molecular methods using samples collected from presumably healthy adult owls. In North American owls, the only morphologically distinct *Leucocytozoon* is *L. danilewskyi*, but evidence suggests that there are at least two cryptic species or subspecies, highlighting the need to genetically characterize these parasites to understand their natural history and relative importance in morbidity and mortality (Ishak et al., 2008).

Here we describe haemosporidian infections including the pathology and molecular characterization of parasites in seven great horned owls from two geographic locations in the United States, Louisiana and California, in spring 2015, 2016, and 2017. The clinical findings for one surviving owl and three owls prior to death are also described.

2. Case reports

2.1. Louisiana, USA

A fledgling great horned owl (A) was found on the ground in May 2015 by a private citizen in Livingston Parish, LA. The owl died overnight with no obvious signs of trauma or other external abnormalities. The carcass was brought to the Louisiana Department of Wildlife and Fisheries (Baton Rouge, LA) who submitted it to the Southeastern Cooperative Wildlife Disease Study (SCWDS; Athens, GA) for necropsy.

Owl A was a female in fair nutritional condition. Aside from general pallor of all tissues, particularly the subcutis and lungs, no other significant gross lesions were detected. Representative organ samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Five micron thick sections were placed on glass slides and stained with hematoxylin and eosin. Additional ancillary tests included testing for avian influenza viruses by polymerase chain reaction (PCR) assay for the matrix gene, West Nile virus by virus isolation, herpesviruses by PCR, and anticoagulant rodenticides by a toxicological screen of liver tissue. Apart from a trace amount of brodifacoum, none of the aforementioned pathogens or compounds were detected.

Microscopic examination revealed multifocal, variably-shaped and often lobulated, megalomeronts ranging between 30 and 50 μm in diameter and 30 to 100 μm in length in the liver, spleen, kidney, and heart (Fig. 1). The cells surrounding the meronts often lacked cellular detail and contained scant nuclear debris, particularly in the spleen, heart, and liver (Fig. 1A–C). The meronts were also associated with occasional hemorrhage, as well as rare clusters of brown, globular intracytoplasmic and extracytoplasmic pigment interpreted as hemosiderin. Round, basophilic host cell nuclei (residual bodies) were often visible within megalomeronts. Variable numbers of lymphocytes, plasma cells, and macrophages were observed forming concentric rings around the meronts. The meronts contained numerous merozoites approximately 1–2 μm in length within organized cytomeres, which is morphologically consistent with that reported in *L. danilewskyi* infection (Khan, 1975; Valkiūnas, 2005) (Fig. 1D). Less than 1% of the leukocytes within the vessels of the lung had expanded, pale basophilic cytoplasm, eccentric nuclei, and intracellular, pigmented sporozoites (Table 1).

2.2. California, USA

Two sibling, nestling great horned owls (B, C) were found on the ground in Sacramento County, CA in late March 2016 and admitted to the University of California, Davis Small Animal Clinic (Davis, CA). Physical examination of both owls revealed dehydration, 3/9 body condition score, poor appetite, active regurgitation, and no evidence of trauma. Owl B was anemic (hematocrit 6%) and hypoproteinemic (total protein 2.6 g/dL). Fresh whole blood and wedge smear technique were used to prepare blood smears, which were stained with a modified Wright stain (Aerospray Hematology Pro, Wescor, Inc.). Abundant round to elongate, pale basophilic inclusions in immature erythrocytes and leukocytes, that mildly distorted the cells and displaced the nuclei peripherally (Fig. 2A and B), were present in blood smears. Immature gametocytes in mature erythrocytes measured between 0.9 and 1.7 μm in diameter. Gametocytes in roundish host cells measured between 6.5 and 14.2 μm in length, and gametocytes in fusiform host cells measured between 21.1 and 26.4 μm in length. In addition to the elongated nucleus of the fusiform host cells that are in close approximation to the primarily immature gametocytes in immature erythrocytes, these characteristics were morphologically consistent with *Leucocytozoon* sp. (likely *L. danilewskyi*) (Valkiūnas, 2005). Due to poor prognosis, owl B was euthanized and a necropsy was performed. Owl C survived to release (Table 1).

Owl B was a male in poor nutritional condition. Similar to owl A, generalized pallor of the skeletal muscles and viscera was present (anemia). Approximately ten multifocally confluent, roughly parallel, dark red to black tracts extended from the capsular surface into the parenchyma on the medial surfaces of the right and left liver lobes. These tracts were 2–6 mm in diameter and up to 1.5 cm long, and some tracts contained small amounts of yellow, soft, granular material.

Histopathologically, haemosporidian megalomeronts were observed in numerous tissues, including the liver, kidneys, spleen, lungs, proventricular glands, small intestinal mucosa, heart (myocardium and subepicardium), thyroid, pancreas, bursa of Fabricius, and marrow spaces of the scleral ossicles similar to owl A. Megalomeronts ranged from 80 to 140 μm in diameter, and were often subdivided into 3–6 discrete cytomeres. The nuclei of affected host cells were often enlarged up to 40 μm in diameter. Highest concentrations of megalomeronts were seen in the liver, kidneys, and scleral ossicles. Associated inflammation was generally absent or minimal. In the kidneys a mild, mixed inflammatory cell infiltrate was present that multifocally expanded the interstitium adjacent to megalomeronts. This infiltrate consisted primarily of histiocytes and lymphocytes with fewer granulocytes. In the liver, sinusoids were diffusely, mildly expanded by increased numbers of mononuclear inflammatory cells that occasionally contained indented or peripheralized nuclei. Multifocal, random foci of coagulative necrosis associated with hemorrhage were scattered throughout liver sections, similar to owl A. One similar focus was present in a section of lung.

At admission, owl C's red blood cell count was 1.5 M/μL, hematocrit