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Diversity and prevalence of hemoparasites of wading birds in southern Florida, USA

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ABSTRACT

Relatively few studies on hemoparasites have been conducted on wading birds in the families Ardeidae and Threskiornithidae (order Pelecaniformes), especially in the United States. In this study, we obtained baseline data on the prevalence and genetic diversity of haemosporidian parasites in wading birds opportunistically sampled from southern Florida, USA. We detected blood parasites in White Ibis (*Eudocimus albus*), Glossy Ibis (*Plegadis falcinellus*), Green Heron (*Butorides virescens*), and Roseate Spoonbill (*Platalea ajaja*) with several novel host-parasite relationships. Infected birds had low parasitemias (average 0.77%, range 0–4%) suggesting that infections were chronic. Despite the low sample sizes for several of our sampled species, these data highlight the diversity of parasites in this understudied group of birds and suggest that additional studies are needed to investigate the potential impacts of these parasites on their health, especially since southern Florida is becoming increasingly urbanized which can alter parasite transmission or host susceptibility.

1. Introduction

Vector-borne, protozoan parasites in the order Haemosporida (genera *Plasmodium, Haemoproteus, Leucocytozoon*, and *Fallisia*) can cause significant morbidity and mortality in some species of birds. Generally, haemosporidian infections often are well tolerated by their natural bird hosts; however, young birds, birds that are outside their normal range, or birds in areas where parasites are introduced are more likely to develop clinical disease (Dawson and Bortolotti, 2000; Valkiūnas, 2005). Common examples include mortality of captive penguins due to *Plasmodium* spp. circulating in native bird populations around the zoo and the significant impact of *P. relictum* after it was introduced to avifauna in Hawaii (Herman et al., 1968; Vanstreels et al., 2014; Samuel et al., 2015). In their natural hosts, haemosporidian parasites generally establish long-term infections and the long-term consequences of these infections have been extensively studied. Field and experimental studies have detected significant impacts of some haemosporidian chronic infections on birds including reduced reproductive success, host fitness, increased stress and disease susceptibility (Arriero et al., 2008; Knowles et al., 2010; Lachish et al., 2011; Dhondt et al., 2017). In addition, Asghar et al. (2015) recently reported that Great Reed Warblers (*Acrocephalus arundinaceus*) infected with *Plasmodium ashfordi* had shorter telomere lengths, which ultimately was correlated with decreased life spans. Collectively, these data highlighted the potential for these parasites to exert a significant population level impact on a host species, even in cases where they might not cause acute mortality.

Although numerous studies have investigated hemoparasites of Passeriformes and Anseriformes, relatively few studies on hemoparasites have been conducted on birds in the families Ardeidae and Threskiornithidae (currently in the order Pelecaniformes, but

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historically in the order Ciconiiformes). Most surveys of these families have been outside of North America where *Haemoproteus plataleae* has been reported from Eurasian Spoonbill (*Platalea leucorodia*), Glossy Ibis (*Plegadis falcinellus*), Australian White Ibis (*Threskiornis molucca*) and Red-naped Ibis (*Pseudibis papillosa*), *Haemoproteus pelouroi* from Hadada Ibis (*Bostrychia hagedas*) and African Sacred Ibis (*T. aethiopicus*), *Leucocytozoon leboeufi* (=*L. ardeae*) from Australian White Ibis, and *Fallisia neotropicalis* from Green Ibis (*Mesembrinibis cayennensis*) and Scarlet Ibis (*Eudocimus ruber*) (Valkiūnas, 2005).

In the United States, the White Ibis (*Eudocimus albus*) is the only member of the Threskiornithidae that has been sampled for blood parasites. Based on blood smear analysis, *H. plataleae* occurs in high prevalence in adult White Ibises and this parasite has been reported in five counties in Florida (Forrester, 1980). Recently, an uncharacterized *Plasmodium* sp. was detected in a single White Ibis from southern Florida by polymerase chain reaction (PCR) testing (Bryan et al., 2015). In contrast, despite limited testing, several hemoparasites have been reported from sympatric North American species of egrets and herons (family Ardeidae) including two species of *Plasmodium* (*P. relictum* and *P. elongatum*), two species of *Haemoproteus* (*H. herodiadis* and an unnamed *Haemoproteus* sp.), and one species of *Leucocytozoon* (*L. leboeufi*) (Telford et al., 1992; Beadell et al., 2006). None of these studies used a combination of morphological data and molecular analysis for identification which can lead to an underestimation of diversity.

Although few studies have examined members of the Ardeidae for blood parasites, these studies have focused on herons and egrets which were infected with multiple genera of parasites. Therefore, ibises and their close relatives likely have an unrecognized diversity of blood parasites. Therefore, our goal was to determine the prevalence, parasitemias, and diversity of hemoparasites in opportunistically sampled Pelecaniformes of southern Florida.

2. Materials and methods

Sample collection. From 2013 to 2016, blood samples were collected throughout the year from wading birds admitted to rehabilitation centers in urbanized areas of Lee and Broward Counties (26° 26′37.6″ N 82° 06′55.52″ W and 26° 05′04.20″ N 80° 08′46.53″ W, respectively). Birds exhibited a variety of conditions including lacerations, fractures, and dehydration. In May 2014, wading bird chicks were hand captured from two nesting sites from a natural area in Broward County (26°11′41.02″N 80°31′28.29″W and 26° 7′19.35″N 80°32′29.09″W) as described by Hernandez et al. (2016). Blood samples were collected from the jugular or medial metatarsal vein into heparinized microtainer® tubes (Beckton Dickinson, Franklin Lakes, New Jersey) and two thin blood smears were immediately prepared, dried, and fixed in methanol. Remaining blood was frozen at −20C until PCR testing. In some cases, birds admitted to rehabilitation centers died prior to blood collection; therefore, only clotted blood was available for PCR testing. All capture and sampling techniques were reviewed and approved by the University of Georgia's IACUC (#A2013- 10–016).

Genetic characterization. DNA was extracted from whole blood samples (10 μl) using a Qiagen DNeasy blood extraction kit per the manufacturer's instructions (Qiagen, Valencia, California). Nested PCR was used to target a 480 base pair (bp) fragment of the mitochondrial cytochrome *b* gene of *Haemoproteus* and *Plasmodium* and a 478 bp fragment of *Leucocytozoon*, as described by (Hellgren et al., 2004; Waldenström et al., 2004). Secondary PCR products were electrophoresed in 2% agarose gels stained with ethidium bromide. Amplicons were excised from the gel and purified using the Qiagen QIAquick gel extraction kit (Qiagen) and sequenced at the Georgia Genomics Facility in Athens, GA using the Sanger method. Sequences were analyzed and aligned using Sequencher (v5.0) and then compared with related sequences in the GenBank and the MalAvi databases to determine related haplotypes. The GenBank accession number for the novel sequence of *H. plataleae* is MF536976.

DNA from blood samples from ducks infected with *H. nettionis* or *L. simondi* were used as a positive control in each set of PCR reactions. To prevent and detect contamination, DNA extraction, primary and secondary amplification, and product analysis were done in separate dedicated areas. A negative water control was included before and after each set of 10–12 extractions. Additional negative water controls were included in each set of primary and secondary PCR reaction sets.

Blood Smear Analyses. Thin blood smears were stained with a modified Giemsa stain (Dipquick, Jorgensen Laboratories, Inc., Loveland, CO). To estimate parasitemias, approximately 20,000 erythrocytes were examined for blood smears determined to be positive as suggested by Godfrey et al. (1987). If no parasites were observed during this initial scan, the smear was examined for another 5 min (generally this would result in another 20,000 erythrocytes or more examined). The parasites were morphologically identified using a published key (Valkiūnas, 2005) or by examination of specific parasite descriptions (e.g., Tostes et al., 2017).

3. Results

Samples were collected from six species of wading birds: White Ibis, Glossy Ibis, Roseate Spoonbill (*Platalea ajaja)*, Green Heron (*Butorides virescens*), Tricolored Heron (*Egretta tricolor*), and Great Blue Heron (*Ardea herodias*) (Table 1). In Lee County, three species (White Ibis, Glossy Ibis, and Green Herons) were positive using the *Plasmodium/Haemoproteus* PCR assay (Table 1). At the Broward County rehabilitation center, 5/14 (36%) White Ibises were PCR positive with the *Plasmodium/Haemoproteus* PCR assay. Among the chicks sampled in Broward County, only one Roseate Spoonbill chick estimated to be 20–30 days old was PCR positive (Table 1). The remaining chicks were negative; the White Ibises were estimated to be either 8–12 days (n = 2) or 29–33 days old (n = 2) and the Glossy Ibis chick was 8–12 days old.

Overall, based on sequence analysis, two *Plasmodium* spp. and one *Haemoproteus* sp. were detected (Table 2). One Green Heron and the Roseate Spoonbill were infected with *Plasmodium elongatum* (haplotype pGRW06/MD-2011) (Table 2). The Glossy Ibis *Plasmodium* sp. sequence was identical to lineage pMYCAME02 (also called CMV-2012). All of the positive White Ibises and one Green Heron were infected with a novel *Haemoproteus* haplotype (designated hWHIB01) that was 98.6% similar (477/484bp) to a *Haemoproteus* sp. from West Africa (hCELEC01/haplotype WAH8).

Blood smears were only available for 17 birds, nine of which were PCR-positive birds. No parasites were observed in PCR negative birds but they were observed in eight of nine PCR-positive birds. In general, the parasitemias were low and ranged from 0 to 4% (average of 0.77%). The *Haemoproteus* haplotype found in the White Ibis was morphologically identified as *H. plataleae* (Table 2). The parasites in the *Plasmodium*-positive Green Heron were morphologically consistent with *P. elongatum* with gametocytes that measured approximately 16 μm × 2.5 μm and did not displace the host cell nucleus. The Glossy Ibis infected with *Plasmodium* had the highest parasitemia detected in the study (4%); ~10% of infected erythrocytes contained 2–4 parasites (Fig. 1). The parasites were identified as a *Plasmodium* (*Novyella*) sp. and shared some morphological characteristics reported for *P. paranucleophilum* as described by Tostes et al. (2017). However, because no phanerozoites were observed in circulating blood cells, few gametocytes were observed, and the parasite nuclei stained poorly, we were