Identifying Avian Haemosporidians in Turkey Vultures and Black Vultures from South Carolina

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Introduction

Avian haemosporidians are intraerythrocytic parasites that collectively cause avian malaria. Although most chronic infections are considered innocuous in the short-term, a recent study suggests that long-term effects of infections can decreased life spans (Valkiunas, 2005; Asghar et al., 2015).

Vultures in the genera *Cathartes* and *Coragyps* are widespread throughout the New World and play important roles in the ecosystem by scavenging. Several haemosporidians (i.e., *Haemoproteus catharti, Plasmodium elongatum,* and *Leucocytozoon* sp.) have been reported from Turkey Vultures (*Cathartes aura*), but only a single *Plasmodium* infection has been reported in a Black Vulture (*Coragyps atratus*) from Florida (Webb et al., 2005). To date, no molecular data is available for any malarial parasite in any vulture species.

OBJECTIVES:

1. **To determine prevalence of blood parasites of Turkey and Black Vultures from South Carolina**
2. **To conduct genetic and morphologic characterization work on blood parasites infecting Turkey Vultures and Black Vultures**

Savannah River Site

Methods

Vultures were trapped on several different sites at the Savannah River Site (SRS) in Aiken, SC using net blasters. A blood sample was collected a blood slide was made immediately. Remaining blood was frozen until PCR analysis. Blood smears were air dried, fixed in methanol, and stained with modified Giemsa. A minimum of 20,000 erythrocytes were examined for parasites. A subset of positive samples was tested for *Haemoproteus* and *Plasmodium* using nested PCR for the mitochondrial cytochrome *b* (*cyt b*) gene (Waldenström et al., 2004; Hellgren et al., 2004).

Amplicons were extracted from the gel, purified, and bi-directionally sequenced. Sequences were analyzed and aligned with related sequences using the multisequence alignment tool in MEGA program (Kumar et al. 1993). Phylogenetic analysis using the neighbor-joining algorithm using the Kimura 2-parameter model and maximum parsimony using a heuristic search was conducted in MEGA.

Results

Sixteen of 37 (43%) Turkey Vultures were positive (43%) for blood parasites. All were morphologically identified as *H. catharti* (Figure 1, Table 1). None of the 51 Black Vultures were positive for blood parasites. The difference in prevalence between the species was significant (p value<0.0001). No difference in prevalence between site, sex, and age categories was found (Figs. 2-4). Parasitemias were low in all infected birds (Fig. 5).

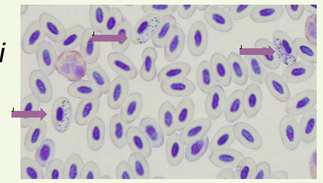


Figure 1. Microgametocytes (arrows) from Turkey Vulture

Figure 2. Prevalence of *H. catharti* in turkey vultures by site

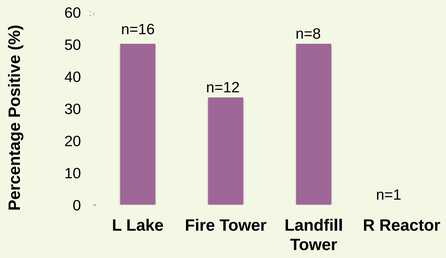


Figure 3. Prevalence of *H. catharti* in turkey vultures by sex

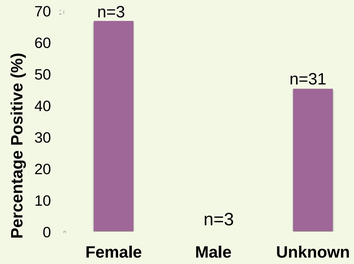


Figure 4. Prevalence of *H. catharti* in turkey vultures by age

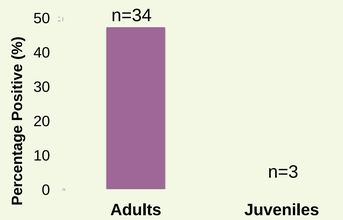


Figure 5. Parasitemias of *H. catharti* in turkey vultures

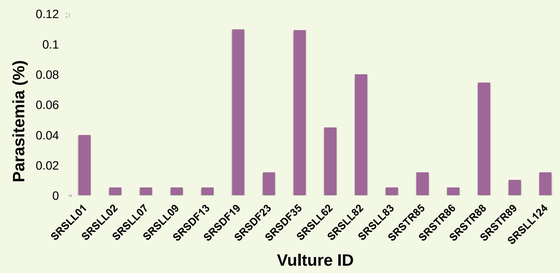


Table 1. Comparison of morphological data from current study with data from Greiner et al. 2011

	Macrogametocytes		Microgametocytes	
	Current Study (n=15)	**Greiner (n=20)**	**Current Study (n=21)**	**Greiner (n=3)**
Gametocyte Length	14.1 ± 1.3 (12-16)	15.4 ± 1.1 (14-18)	13.5 ± 1.2 (11-16)	14.5 ± 0.6 (14-15)
Gametocyte Width	3.8 ± 0.4 (3.0-4.5)	3.4 ± 0.5 (2.5-5)	3.9 ± 0.6 (2.5-5)	3.7 ± 0.6 (3-4)
Nuclear Displacement Ratio (NDR)	0.62 ± 0.2 (0.375-1)	0.59 ± 0.2 (0.2-1)	0.50 ± 0.1 (0.2-0.7)	0.5 ± 0.2 (0.3-0.7)
Infected RBC Length	14.2 ± 0.6 (13-15)	15.4 ± 1.0 (14-17)	14.3 ± 1.1 (13-16)	15.2 ± 1.3 (14-16.5)
Infected RBC Width	8.3 ± 0.8 (7-10)	7.7 ± 0.7 (7-9)	8.2 ± 0.9 (7-10)	7.8 ± 0.8 (7-8.5)
Uninfected RBC Length	Current: 14.2 ± 0.7 (13-16) (n=35)		Greiner: 14.2 ± 0.8 (13-16) (n=10)	
Uninfected RBC Width	Current: 8.0 ± 0.6 (7-9) (n=35)		Greiner: 7.9 ± 0.5 (7-9) (n=10)	
Pigment Granule Number	28.5 ± 6.4 (14-38)	24.4 ± 7.0 (19-33)	24.8 ± 4.6 (17-34)	18.0 ± 7.0 (11-25)
Parasite in contact with host cell nucleus?	Usually yes	Not usually	Usually yes	Not usually

Nested PCR targeting the cytochrome b gene was used to test a subset of blood. Nine slide-positive Turkey Vultures were also PCR positive and seven slide-negative Black Vultures were PCR negative.

Based on cytB gene sequence analysis, *H. catharti* was included in a distinct clade with two other sequences from Genbank (Figure 6). An unpublished *H. catharti* sequence from a turkey vulture from California was 99.9% identical to our sequence.

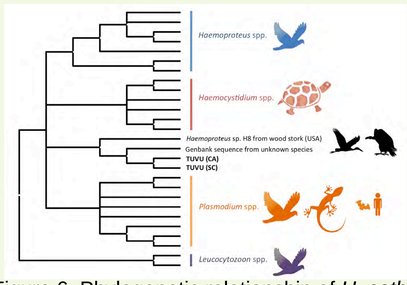


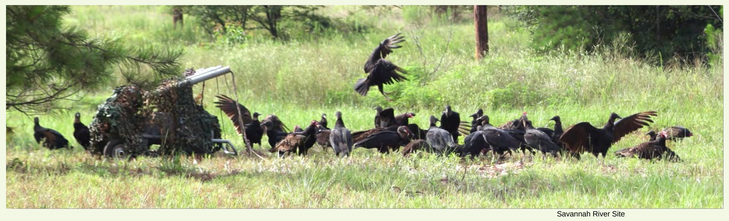
Figure 6. Phylogenetic relationship of *H. catharti* from turkey vultures from SC and CA.

Discussion

The prevalence of *H. catharti* in Turkey Vultures (43%) at SRS is higher than previous reports of *Haemoproteus* in turkey vultures from various sites in the southeastern US (9-18%) (Williams and Bennett, 1978; Wetmore, 1941; Webb et al., 2005) but lower than a small study in Panama (75% of 4 vultures) (Darling, 1912). No blood parasites were detected in Black Vultures. Although a previous study found a single Black Vulture with a co-infection of *Plasmodium* sp. and *Leucocytozoon* sp. (Forrester and Spalding 2003), other studies have not found blood parasites in Black Vultures (Webb et al., 2005). While Black and Turkey Vultures have similar habitat requirements and anatomy, their behavior often differs (DeVault, 2004). Additional work is needed to understand the lack of blood parasites in Black Vultures.

The partial cytb sequences from 8 turkey vultures were identical and only varied by a single base from a sample from a turkey vulture from California (Perkins, unpublished data). Phylogenetically, these sequences formed a unique clade which was distinct from the genera *Haemoproteus* and *Plasmodium*. These data suggests that *H. catharti* and a blood parasite of wood storks belong in a new genus.

This study documents the first genetic work done on malarial parasites in vultures and confirms, by PCR, the low prevalence of blood parasites found in Black Vultures at the SRS. Further genetic characterization work targeting different genes will be used to determine if this parasite is indeed a separate genus of blood parasites.



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Acknowledgements

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