Blood-borne parasites in the Black Vulture *Coragyps atratus* in northwestern Costa Rica

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Abstract

Blood parasites of 17 Black Vultures (*Coragyps atratus*) were surveyed. The birds were captured from two sites- a garbage dump in Miramar, Puntarenas and a beach in Cuajiniquil, Guanacaste, of Costa Rica. Two blood smears were made per bird, stained, and examined for *Haemoproteus*, *Leucocytozoon*, *Trypanosoma*, *Plasmodium* and microfilaria presence. Both *Plasmodium* and microfilaria were found, with an overall prevalence of blood parasites of 70.6% (76.92% in Puntarenas, 50% in Guanacaste). The overall prevalence of *Plasmodium* was 46.2% and of microfilaria 58.8%. Chi-squared tests revealed capture location had no effect on presence of *Plasmodium*, while microfilariae were significantly more common at the Puntarenas site. No *Haemoproteus*, *Leucocytozoon*, or *Trypanosoma* were observed at either site.

Resumen

Los parásitos de sangre en 17 Zopilotes Negros (*Coragyps atratus*) fueron estudiados. Los zopilotes fueron capturados en dos sitios: el relleno sanitario en Zagala, Puntarenas, y en Cuajiniquil, Guanacaste, en Costa Rica. Dos frotices sanguíneos fueron hecho por ave, y los fueron teñido y examinado en busca de *Haemoproteus, Leucocytozoon, Trypanosoma*, *Plasmodium* y microfilaria. Tanto *Plasmodium* y microfilaria fueron detectado, con una preponderancia total de parásitos de sangre de 70.6% (76.92% en Puntarenas, 50% en Guanacaste). La preponderancia total de *Plasmodium* fue 46.2%, y de microfilaria 58.8%. Una prueba de chi cuadrado reveló que el sitio de captura no tuvo efecto en la presencia de *Plasmodium*, mientras que las microfilariae fueron considerablemente más comunes en Zagala. Ningún *Haemoproteus*, *Leucocytozoon*, o *Trypanosoma* fueron observado en ningún sitio.

Introduction

The Black Vulture (*Coragyps atratus*) is a large neotropical resident species of new world vulture, ranging from the southern United States to central Chile and Uruguay. While Black Vultures have been known to kill weak or moribund animals and newborn livestock, they are primarily scavengers and carrion feeders, and as such provide an important ecosystem service. (Buckley 1999).

Blood parasites can cause morbidity and death in many species of birds (Webb *et al.* 2005, Ritchie *et al.* 1994, Thrall *et al.* 2004). While parasitemia is commonly studied, there is a paucity of data available on the prevalence of blood parasites in birds in Costa Rica, especially with regard to birds of prey in general and Black Vultures in particular. Blood parasite studies are generally conducted by mist-netting, resulting in a sampling bias against types of birds not likely to be caught in mist nets.

This study seeks to survey the type and prevalence of blood parasites in Black Vultures in the Mirasol, Puntarenas and Cuajiniquil, Guanacaste areas. Extrapolating from data regarding other species in the same zones and conspecifics in higher latitudes, the most likely parasites to be found in this cathartid in the Puntarenas and Guanacaste areas are species of the protozoan genera *Haemoproteus, Leucocytozoon, Trypanosoma* and *Plasmodium* and microfilariae of filarial nematodes, (Valkiunas et al 2004, Young *et al.* 1993, Benedikt *et al.* 2009, Webb *et al.* 2005).

Haemoproteus spp. are parasitic protozoa, common in many wild birds. Under normal circumstances, *Haemoproteus* are considered non-pathogenic, but high parasitemias can cause anemia, splenomegaly, hepatomegaly, and pulmonary edema, and have also been shown to reduce reproductive fitness of some birds in the wild (Merino *et al.* 2000). They are transmitted by Culicoides and louse flies (Ritchie *et al.* 1994). *Haemoproteus* are found in peripheral blood only as gametocytes, and range in form from developing rings to elongate mature gametocytes that partially encircle the nucleus of an erythrocyte, generally occupying greater than half of the cytoplasmic volume of the host (Thrall *et al.* 2004).

Parasites of the *Plasmodium* genus vary greatly in pathogenicity, with some species highly pathogenic and responsible for avian malaria (Atkinson *et al*. 1991). Clinical signs are most common immediately after infection and include anemia, dyspnea, weakness, anorexia, and death, but many birds serve as asymptomatic carriers. Mosquitoes are the primary vectors (Atkinson *et al*. 1991, Ritchie *et al*. 1994). Gametocyte, schizont and trophozoite stages can be found within erythrocytes, thrombocytes, and leukocytes (Thrall *et al*. 2004).

The genus *Leucocytozoon* is composed of parasitic protozoa. While infected individuals generally exhibit no clinical signs at low levels, symptoms in individuals with greater parasitemias include anemia, dyspnea, and death (Ritchie *et al*. 1994). Along with *Haemoproteus*, they have been implicated in lowered reproductive success in some birds, although they primarily effect stressed, young, and immunonaive birds. (Atkinson *et al*. 1991, Merino *et al*. 2000). They are identified by dark-staining macrogametocytes or light-staining microgametocytes, which cause the cell to elongate and distend, making host cell identification difficult (Thrall *et al*. 2004). *Leucocytozoon* spp. are transmitted by Culicoides (Ritchie *et al*. 1994).

Trypanosomes are extracellular flagellated parasites categorized as minimally pathogenic, transmitted by black flies and Culicoides (Ritchie *et al*. 1994).

Microfilariae are immature filarial nematodes and impossible to identify without the adult. Adult filarial nematodes are commonly found in air sacs, subcutaneously, or in body cavities, but never the blood stream (Thrall *et al.* 2004). Most are considered apathogenic; however, adult filarial worms can cause severe problems, such as tendonitis, pericarditis, and asphyxiation (Ritchie *et al.* 1994).

Materials and Methods

Black Vultures were caught by the legs with a rope noose, using fresh meat as bait at two locations- the Zagala landfill in Miramar, adjacent to the Pan-American highway in Puntarenas, and the beach near the town of Cuajiniquil, Guanacaste. With the help of José Chan and the workers of the Zagala landfill, 13 vultures were sampled from the landfill, and four from the Cuajiniquil beach. 0.1 mL of blood was taken with a 30-

gauge syringe from the basilic vein, located on the ventral aspect of the elbow. Two blood smears were made per bird and air-dried in the field, before being fixed with 100% methanol for one minute. In the laboratory, the fixed slides were stained for 20 minutes in 5% Giemsa stain solution, rinsed with distilled water, and allowed to air dry. Each of the two blood smears made per bird was inspected for *Haemoproteus, Leucocytozoon, Trypanosoma,* and *Plasmodium,* as well as microfilariae. Presence and density of *Haemoproteus, Leucocytozoon,* and *Plasmodium* were determined by counting 20 replicates of 100 blood cells on each of the two slides at 1000x magnification and the infection was scored as number of infected cells/2,000 erythrocytes, according to protocol set by Godfrey *et al.* (1987). The entire side was scanned at 400x magnification for presence or absence of microfilaria and *Trypanosoma*. Hemoprotozoans were identified to genus level, due to difficulties in species identification at low parasitemia with few life stages present. Density of microfilariae was not measured - no standardized protocol for evaluation of microfilaria density exists. Microfilarial infections demonstrate high levels of periodicity in the blood stream, with the level of periodicity varying greatly by species (Kloss *et al.* 2003, Nogami *et al.* 2000). Because sampling occurred at an unknown point in this period, and species was unknown, only presence/absence could reasonably be determined.

Results

The overall prevalence of blood parasites from both sites was 70.6%, with the prevalence of *Plasmodium* at 35.3% and microfilaria at 58.8%. No *Haemoproteus, Leucocytozoon,* or *Trypanosoma* were observed in the birds from either the landfill at Zagala or Cuajiniquil. *Plasmodium* (Figure 1) was found in 5 individuals from the landfill (38.5%) and one from Cuajiniquil (25.0%). All *Plasmodium* infections ranged between 0.025% and 0.1% of observed infected cells. Microfilariae (Figure 2) were observed in nine individuals from the landfill (69.2%) and one from the Cuajinquil beach (25.0%), for an overall prevalence of 58.8%. Overall parasite prevalence in birds captured at the landfill was 76.92%, and prevalence at the Cuajiniquil beach site was 50.00%.

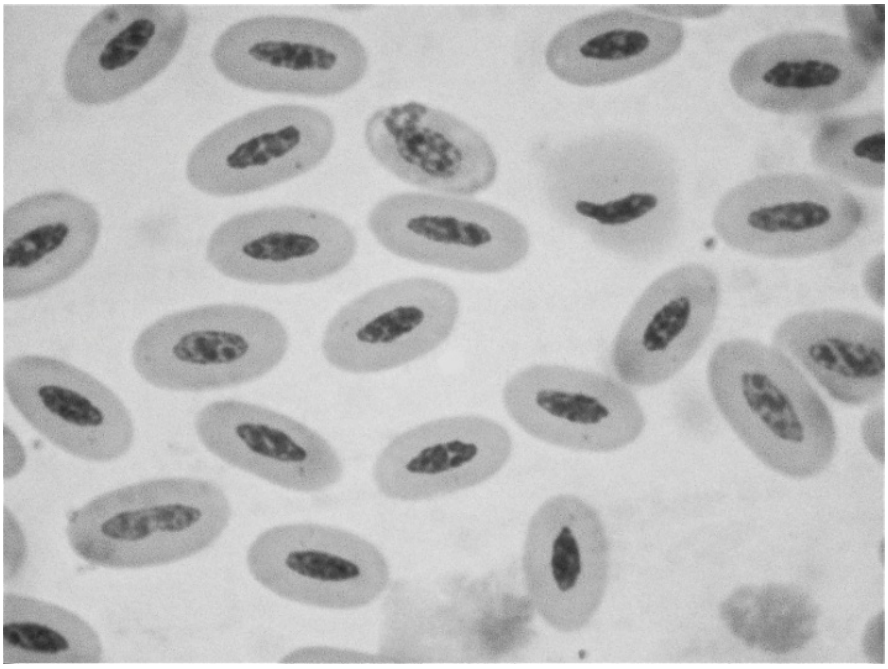

Figure 1: *Plasmodium* infection in Black Vulture erythrocyte.

Figure 2: Microfilarium in Black Vulture blood smear.

There was no association between the site and prevalence of *Plasmodium*, as indicated by a chi-squared test ($\chi^2 = 0.243$, df = 1, p = 0.6223). However, there is a loose association between the sample location and the presence of microfilariae, ($\chi^2 = 2.471$, df = 1, p = 0.1160) with microfilariae found to be more common at the landfill. Presence of one type of parasite was found to be not associated with the presence of the other ($\chi^2 = 0.442$, df = 1, p = 0.5060).

Table 1: Parasite Prevalence by Site

	Zagala	Cuajiniquil	Overall
Plasmodium	38.5%	25.0%	35.3%
Microfilaria	69.2%	25.0%	58.8%
Overall	76.9%	50.0%	70.6%

Discussion

Because no surveys have been conducted for blood parasites of Cathartidae in Costa Rica, the significance of these results is difficult to assess. The only comparisons that can be made are between much less focused studies located in the same area, which looked at many different species of mostly smaller birds, and included no Cathartids. In comparison to these studies, a very high rate of infected individuals was found in the vultures sampled here. Valkiunas *et al.* (2004) sampled diverse bird genera in the Área de Conservación Guanacaste and found 12.4% were infected with blood parasites- species of *Haemoproteus* (4.8% prevalence), *Plasmodium* (0.6%), *Leucocytozoon* (0.3%), *Trypanosoma* (2.0%), and microfilariae (7.6%) were all observed. Young *et al.* (1993) sampled many bird genera in the

Monteverde area and found an overall prevalence of 10.6%, with *Haemoproteus* (9.4% prevalence), *Plasmodium* (0.4%), *Leucocytozoon* (0.4%), *Trypanosoma* (0.2%), and microfilariae (1.0%) all being observed. However, neither of these studies looked at Black Vultures, or any cathartids, and the rate of infection for individual species varied between zero and 100% of individuals affected. While no conspecific comparison can be made, these studies indicate that the Black Vultures sampled at both sites demonstrate a relatively high rate of parasitemia when compared with most bird species in the area.

The cause of this unusually high level of parasitemia is uncertain. Both sites have relatively high densities of Black Vulture, with the landfill supporting a population of between one and two thousand, which could increase the ease with which infections spread. It is also possible that Black Vultures, as a species, are more likely to be infected by *Plasmodium* and microfilaria, due to life history or physiology. Lack of correlation between location and *Plasmodium* infection implies a constancy of infection rates across populations, suggesting that the species as a whole is more tolerant to infection, regardless of location. This is contrasted by the significant difference between rates of infection of microfilaria, which bespeaks environmental factors, such as vector abundance, influencing the infection rates. Further research could be conducted by sampling birds from populations of varying density to determine the rate of infection when the birds have differing levels of conspecific contact. Concurrent with this, a survey of insect vectors in the various sites would testify to any correlation between vector abundance/type and rates of parasitemia. Correlation or lack thereof between vulture density, vector abundance/type and rates of parasitemia could clarify whether the higher observed rates here were due to species life history, physiology, or vector abundance. More likely it is a combination of the above factors, as suggested in other avian species (Martínez-Abraín 2004).

All observed *Plasmodium* infections were low intensity according to the standards set by Valkiunas (2004), which indicates that the birds in questions would be sub-clinical (demonstrating no symptoms of infection) and would most likely not experience any adverse effects from the parasitemia (Ritchie *et al*. 1994, Thrall *et al*. 2004). Identification of *Plasmodium* from blood stages alone is difficult at best, even in an individual with a severe infection and all developmental stages present, and could not be determined in this study. Identification to species level would provide valuable information, since only a fraction of the 34 avian *Plasmodium* species recognized by Bennett *et al*. (1993) are responsible for avian malaria. Due to the low levels of parasitemia, none of the birds sampled here would be in danger from their infections.

While the sampled birds seem to be healthy regardless of parasite carriage, the landfill could be serving as a reservoir and breeding ground for pathogenic species of *Plasmodium*. As a reservoir, it would serve as a constant source population of *Plasmodium* spp., to be carried by vectors to other bird populations, including species which may have a greater susceptibility to *Plasmodium* infection. More importantly, changes in global climate patterns are altering the ranges of both bird species and insect vectors (DEFRA 2005, Epstein 2001). This increases the chances of immunonaïve birds being exposed to *Plasmodium* spp., which has been shown to have catastrophic effects on bird populations, such as the introduction of avian malaria to Hawaii (van Riper 1986). However, if the *Plasmodium* present in the sampled population are of a non-pathogenic species, this impact would be negligible.

Absence of hemoprotozoans of the genera *Haemoproteus, Leucocytozoon,* and *Trypanosoma* is noteworthy though unsurprising. Valkiunas *et al*. (2004) found that of 38 species with parasites present, only three had multiple genera of hemoprotozoans present, and Young (1994) found two out of 12 with multiple protozoan genera present. Finding only hemoprotozoans of the genus *Plasmodium* is thus concordant with previously established patterns. Also in agreement with previous research is the lack of correspondence between presence of one parasite and presence of the other.

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