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## PATTERNS OF *HAEMOPROTEUS BECKERI* PARASITISM IN THE GRAY CATBIRD (*DUMATELLA CAROLINENSIS*) DURING THE BREEDING SEASON

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**ABSTRACT:** We determined the prevalence and intensity of blood parasites in breeding gray catbirds (*DumateLLa carolinensis*) at Killbuck Wildlife Area in Wayne and Holmes Counties, Ohio (USA) from June through August 2000. Of 98 catbirds sampled, 40 (40.8%) had detectable infections of *Haemoproteus beckeri*. Overall prevalence of *H. beckeri* in this population is high relative to that reported in earlier blood parasite surveys of both breeding and migrant catbirds. Mean intensity of *H. beckeri* infection did not vary significantly between young and old birds or among sampling periods. We found no effect of age on prevalence or intensity of *H. beckeri* infection. Older birds were not more likely to be infected than younger birds, despite longer exposure to arthropod vectors. Prevalence varied significantly with season and was highest in June and lowest in August. This pattern also was observed in older birds sampled repeatedly. This seasonal variation may reflect both newly acquired infections and chronic infections relapsing in response to hormonal changes associated with breeding. Evidence of transmission was observed in the single hatching year bird that lacked detectable infection in early summer, but demonstrated a very high intensity infection in late summer. These observations provide supportive evidence that hematozoa infections are acquired on the breeding grounds during the first year of life and relapse during the breeding season in subsequent years.

**Key words:** Blood parasites, *DumateLLa carolinensis*, epizootiology, gray catbird, *Haemoproteus*.

### INTRODUCTION

Protozoan blood parasites have become the focus of a number of avian studies since Hamilton and Zuk (1982) suggested that they may influence plumage brightness in birds and ultimately serve as a mechanism of sexual selection. More recently, interest in avian blood parasites has shifted to their influence on life history traits and energetic trade-offs that result from allocating energy to fighting parasitic infections (Sheldon and Verhulst, 1996). Knowledge of such effects is important for our understanding of the ecology of avian species, especially those experiencing population declines, such as neotropical migrant passerines. Unfortunately, few detailed studies have been conducted on the ecology of blood parasites in migrants, especially on the breeding grounds, and, therefore, we know little about the basic ecology of blood parasites in this group of birds (Valkiunas, 2001).

We studied patterns of prevalence and

intensity of *Haemoproteus beckeri* infections in breeding gray catbirds (*DumateLLa carolinensis*) at Killbuck Wildlife Area in Wayne County, Ohio (USA). The gray catbird is a neotropical migrant passerine bird that breeds in brushy shrub-sapling successional habitat throughout North America (Cimpric and Moore, 1995). Catbirds are especially abundant in the bottomlands of the Killbuck Creek Valley where wetlands with dense thickets dominated by honeysuckle (*Lonicera* spp.) provide optimal breeding conditions. *Haemoproteus* spp. are common protozoan blood parasites of birds and are known to be transmitted by hippoboscids (Diptera: Hippoboscidae; Adie, 1925) and biting midges (Diptera: Ceratopodidae; Fallis and Bennett, 1961). Although various blood parasite surveys have included parasite fauna of gray catbirds (Greiner et al., 1975; Kirkpatrick and Suthers, 1988; Garvin and Remsen, 1993), none have focused on this species during the breeding season when

prevalence and intensity are likely to be high (Janovy, 1966). Here, we report on patterns of prevalence and intensity of *H. beckeri* in gray catbirds on their breeding grounds in central Ohio in relation to age and sampling period.

#### MATERIALS AND METHODS

From June through August 2000, 109 thin blood smears were prepared from 98 gray catbirds captured at Killbuck Marsh Wildlife Area (40°41'N, 81°58'W) in Wayne County, Ohio. Each of the two sampling sites were disturbed abandoned agricultural fields with dense brush surrounded by mixed deciduous forest and marsh. Hatching year (HY) and after hatching year (AHY) birds were captured using Japanese mist nets operated from dawn to approximately noon for 2–3 days each week. Nestlings (L), approximately 10 days old, were removed from nests for bleeding and returned. Birds were marked with serially numbered United States Fish and Wildlife Service bands. Body mass was measured to the nearest 0.1 g using a Pesola® scale (Forestry Suppliers, Inc., Jackson, Mississippi, USA). Blood was collected from the jugular vein of each bird with a 0.1 cc tuberculin syringe fitted with a 28 gauge needle. Thin blood smears were prepared and air dried, then fixed in absolute methanol upon return to the laboratory. Slides were stained in Wright-Geimsa stain (Fisher Scientific, Pittsburgh, Pennsylvania, USA) and examined for parasites by scanning under 1,000× magnification under oil immersion. A minimum of 100,000 erythrocytes was examined per slide. Erythrocyte numbers were determined by estimating the number of erythrocytes in each ¼ field of view and extrapolated to number of cells per entire field of view. Then the appropriate number of fields was read until approximately 100,000 erythrocytes were viewed for each slide. Repeatedly sampled birds were included in the overall prevalence by randomly choosing one sample for each bird. The intensity of infection, reported as number of infected erythrocytes or *Trypanosoma* per 10,000 erythrocytes, was calculated for each bird.

To test for the effect of age on infections, we assigned catbirds to three age categories: nestlings (L) for birds that had not fledged from the nest, HY for post-fledgling birds in their calendar year of hatching, and after hatching year (AHY) for older birds. To test for seasonal variation, we divided the sampling period into 2 week intervals as follows: 7 June–21 June, 22 June–6 July, 7 July–21 July, 22 July–7 August. We used a Chi-square analysis to test for dif-

TABLE 1. Prevalence of *Haemoproteus beckeri* in gray catbirds relative to age.

Age <sup>a</sup>	n	Number infected (%)
L	25	0 (0)
HY	39	23 (59)
AHY	45	24 (53)

<sup>a</sup> L = nestling; HY = hatching year; AHY = after hatching year.

ferences in prevalence of *H. beckeri* infection between age classes and among sampling periods. To test for both the effect of age and sampling period on intensity of infection, we used a Mann-Whitney U-test and a Kruskal-Wallis one-way analysis of variance. Only positive samples were included in analyses of intensity. The nestling group was excluded from all statistical analyses because the prepatent period of *Haemoproteus* is approximately the same as the nestling period, and therefore, infection in nestlings may have been undetectable. Values of  $P < 0.05$  were considered significant. Sample size limitations prevented us from analyzing the effect of age and time on prevalence or intensity of *Trypanosoma* infections. Representative slides were deposited in the US National Parasite Collection (Beltsville, Maryland, USA; Accession numbers 158180142, 158180132).

#### RESULTS

Forty-seven (43.1%) of 109 samples collected from 98 catbirds were positive for *H. beckeri*. To account for birds sampled multiple times, an overall prevalence of 40.8% was calculated by randomly choosing only one sample from each repeated sampled bird. Because subsequent samples from an individual were collected during different periods, for statistical analysis each sample was considered independent. Other hematozoa observed during this time include *Trypanosoma avium* in eight (7.3%) of the samples. All *T. avium* infections were detected in AHY birds.

When comparing HY and AHY birds, we found no effect of age on the prevalence of *H. beckeri* ( $\chi^2 = 0.35$ ,  $df = 1$ ,  $P = 0.55$ , Table 1). Because prevalence did not vary between HY and AHY birds, we combined ages to evaluate the influence of

TABLE 2. Prevalence of *Haemoproteus beckeri* in the HY (hatching year) and AHY (after hatching year) gray catbirds relative to sampling period.

Sampling period	HY		AHY		Total	
	Number	Number infected (%)	Number	Number infected (%)	Number	Number infected (%)
7 June–21 June	0	—	17	16 (93)	17	16 (94)
22 June–6 July	8	4 (50)	12	6 (62)	20	10 (50)
7 July–21 July	19	14 (70)	7	1 (14)	26	15 (68)
22 July–7 August	12	5 (42)	9	1 (17)	21	6 (29)

sampling period on probability of being infected. Prevalence varied significantly through time ( $\chi^2=16.76$ ,  $df=3$ ,  $P=0.001$ , Table 2) and was highest in period 1, 7 June–21 June (94%), and lowest during period 4, 22 July–7 August (29%). Overall, intensity of *H. beckeri* infection appeared to follow a negative binomial distribution with most birds having zero or low intensity infections and few having high intensity infections. Mean intensity of infection did not vary significantly between HY birds (mean=40.99, SD=133.77) and AHY birds (mean=2.65, SD=6.09,  $U=218.5$ ,  $P=0.216$ , Table 3). In HY birds, intensity ranged from <1–750 infected erythrocytes per 10,000 erythrocytes. In AHY birds, intensity ranged from <1–27 infected erythrocytes per 10,000 erythrocytes. Intensity of infection did not vary significantly through time ( $U=0.644$ ,  $df=3$ ,  $P=0.886$ , Table 4). In five of the six catbirds sampled repeatedly, status of infection changed between sampling periods (Table 5). Although sample size limitations prohibited statistical analysis, infection status in five of the six birds changed from detectable to undetectable.

TABLE 3. Mean intensity of *Haemoproteus beckeri* per 10,000 erythrocytes in two age groups of gray catbirds.

Age	Number infected	Mean intensity ( $\pm$ SD)	Range
HY	23	39.36 ( $\pm$ 84.22)	<1–750
AHY	24	5.14 ( $\pm$ 7.96)	<1–27

<sup>a</sup> HY = hatching year; AHY = after hatching year.

## DISCUSSION

The 40.8% prevalence of *H. beckeri* in gray catbirds reported in this study is high relative to other reports of *Haemoproteus* spp. in this species (Greiner et al., 1975; Kirkpatrick and Suthers, 1988; Garvin and Remsen, 1993). The difference between our results and those of the three earlier studies is likely due to seasonal differences in sampling (Bennett et al., 1982). A large literature review by Greiner et al. (1975) and work by Kirkpatrick and Suthers (1988) reported 3.8% and 9.2% prevalence of *Haemoproteus* spp., respectively, in catbirds sampled throughout the annual cycle. Furthermore, Garvin and Remsen (1993) found 0% prevalence in the 59 catbirds sampled in Louisiana during spring migration as birds were en-route from their tropical wintering grounds to their northern breeding grounds.

The relatively higher prevalence of infection reported in our study is likely due to sampling during the breeding season (Weatherhead and Bennett, 1991; Deviche et al., 2001), when relapse of chronic in-

TABLE 4. Intensity of *Haemoproteus beckeri* in gray catbirds, HY (hatching year) and AHY (after hatching year) combined, relative to sampling period.

Sampling period	Total	
	Number infected	Mean intensity ( $\pm$ SD)
7 June–21 June	16	6.5 (9.0)
22 June–6 July	10	20.6 (55.4)
7 July–21 July	15	42.5 (94.1)
22 July–7 August	6	128 (304.6)
Total	47	36.6 (122.0)

TABLE 5. Intensity (number of infected erythrocytes per 10,000 erythrocytes) of *Haemoproteus beckeri* infection in gray catbirds sampled twice.

Bird number	Age	Time period sampled			
		7 June–21 June	22 June–6 July	7 July–21 July	22 July–7 August
1	AHY	2	2.1	—	—
2	AHY	<1	—	0	—
3	L/HY	—	0	—	750
4	AHY	25	—	—	0
5	AHY	16	—	—	0
6	AHY	27	—	—	0

<sup>a</sup> L = nestling; HY = hatching year; AHY = after hatching year.

fections is believed to occur (Herman et al., 1954; Janovy, 1966; Bennett and Fallis, 1960; Greiner, 1975). Relapse, the movement of parasites from the visceral organ tissue to the peripheral circulation, is believed to be triggered by hormonal changes associated with breeding (Applegate, 1970). This mechanism could provide a source for annual initiation of infection in catbird populations if concurrent with seasonal peaks in vector abundance. Our observation that overall prevalence of *H. beckeri* was highest early in the summer, at the beginning of the breeding period, and decreased towards August suggests the role of hormonal changes in initiating relapse. Because catbirds begin breeding in May after returning from their tropical wintering grounds, hormonal changes that cause the onset of breeding behavior are likely to be greatest early in the summer. Moreover, the change in infection status from positive to negative in individual adults sampled both in period 1 and 3 further supports this finding. Similarly, the physiological stress of migration could result in stress responses that suppress the immune system and induce relapse.

Positive samples collected later in the summer likely reflect newly acquired infections and periods of transmission (Herman et al., 1954; Bennett and Fallis, 1960; Greiner et al., 1975). The single bird of the year that was sampled repeatedly demonstrated no detectable infection as a nestling in period 2 and a very high intensity infection as a fledging in period 4 (Table

5). This bird was infected between late June and early August and demonstrates that transmission was occurring during this study, supporting other reports of transmission on the breeding grounds (Bennett et al., 1974).

Geographic variation in vector abundance may also account for the high prevalence reported in this study. Although both known vectors of *Haemoproteus* spp., hippoboscids (Adie, 1925) and species of *Culicoides* (see Bennett and Fallis, 1960), have been observed in association with gray catbirds (Johnson, 1929; Judd, 1959), during this study no hippoboscids were observed on any of the catbirds handled. Therefore, *Culicoides* spp. are the most likely vectors of *H. beckeri* at Killbuck where the wetlands provides ample breeding habitat. Furthermore, *Culicoides arboricola*, an ornithophilic species believed to transmit other species of *Haemoproteus* spp. in passerine birds and forage at catbird nest height (Greiner et al., 1975; Garvin and Greiner, 2003a) was found in a catbird nest collected from Killbuck during the study period (Garvin and Bell, unpubl. data). Accounts of engorged *Culicoides haematopodus* and *Culicoides biguttatus* in catbird nests in Canada (Judd, 1959) are evidence that *Culicoides* are involved in transmission and that some level of transmission is likely to occur at the nest. The absence of detectable infections of *H. beckeri* in nestlings in this study corroborates other studies by Weatherhead and Bennett (1991) and Davidar and

Morton (1993) and may reflect our inability to detect infection through thin blood smears during the pre-patent period. Given that the pre-patent period for *H. beckeri* is likely to be similar to the 12–14 day period known for other species of *Haemoproteus* (Fallis and Bennett, 1961), the 10–12 day old nestlings sampled in this study could have harbored undetectable infections. *Haemoproteus* species have been detected in American kestrel (*Falco sparverius*) nestlings (Dawson and Bortolotti, 1999), however, thus demonstrating that transmission can occur in the nest.

Neither prevalence nor intensity of infection varied with age. Young of the year that were sampled after leaving the nest were no more or less likely to be infected than older birds. This result is in contrast to other studies that report increased prevalence of *Haemoproteus* infection with age (Weatherhead and Bennett, 1991; Davidar and Morton, 1993; Garvin and Greiner, 2003b), probably a result of longer exposure to arthropod vectors (Bennett and Fallis, 1960; Greiner, 1975). Intensity of infection also did not vary with season or age, although our sample size may have been inadequate to detect these differences given that the highest intensity infections were observed in HY birds. The intensities in these individuals probably reflect their immunonaive status and lack of previous exposure to infection relative to adults (Chernin, 1952). These results suggest that infections are likely acquired during the first summer and relapse during the breeding season in subsequent years. However, because immunocompetent AHY birds also may acquire new infections studies of the seasonal abundance of likely vector species are required to more fully understand the degree to which relapse accounts for infections in older birds.

This study provides the first detailed description of temporal patterns of blood parasites in gray catbirds during the breeding season. Although specific for catbirds breeding at our site in central Ohio, our data demonstrate the importance of timing

in sampling for avian hematozoa and provide a basis for planning future work on the influence of *Haemoproteus* on the ecology of gray catbirds and other neotropical migratory passerine birds. Future studies evaluating the impact of the erythrocytic phase of *H. beckeri* infection on gray catbirds should be conducted early in the breeding season when the probability of detecting circulating gametocytes, and the resulting physiological cost of the erythrocytic phase of infection, is likely to be greatest.

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