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Does avian malaria reduce fledging success: an experimental test of the selection hypothesis

Sarah A. Knutie · Jessica L. Waite · Dale H. Clayton

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Abstract Like many parasites, avian haematozoa are often found at lower infection intensities in older birds than young birds. One explanation, known as the "selection" hypothesis, is that infected young birds die before reaching adulthood, thus removing the highest infection intensities from the host population. We tested this hypothesis in the field by experimentally infecting nestling rock pigeons *(Columba livia)* with the malaria parasite *Haemoproteus columbae*. We compared the condition and fledging success of infected nestlings to that of uninfected controls. There was no significant difference in the body mass, fledging success, age at fledging, or post-fledging survival of experimental versus control birds. These results were unexpected, given that long-term studies of older pigeons have demonstrated chronic effects of *H. columbae*. We conclude that *H. columbae* has little impact on nestling pigeons, even when they are directly infected with the parasite. Our study provides no support for the selection hypothesis that older birds have lower parasite loads because parasites are removed from the population by infected nestlings dying. To our knowledge, this is the first study to test the impact of avian malaria using experimental inoculations under natural conditions.

Keywords *Columba livia* · Pigeon · Fitness · Hippoboscid fly · Host-parasite interaction

Introduction

Parasites influence fundamental aspects of the evolutionary ecology of their hosts, such as population dynamics (Anderson and May 1978; Anderson 1979) and life history evolution (Hochberg et al. 1992). The impact of parasites on host fitness depends partly on the age at which hosts become infected. A common pattern in host-parasite interactions is that

Sarah A. Knutie and Jessica L. Waite contributed equally to this work.

S. A. Knutie · J. L. Waite (✉) · D. H. Clayton
Biology Department, University of Utah, Salt Lake City, UT 84112, USA
e-mail: jessi.waite@gmail.com

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younger individuals have higher parasite loads than adults (Gregory et al. 1992; Hudson and Dobson 1997). Sol et al. (2003) considered three hypotheses to explain this pattern. The "selection" hypothesis suggests that highly parasitized juvenile hosts die before they reach adulthood, removing large numbers of parasites from the population. The "immunity" hypothesis suggests that the developing immune system of juveniles is not yet capable of killing parasites, while adults are much more effective at reducing parasite intensity. The "vector exposure" hypothesis suggests that adult behavior reduces their exposure to infected vectors, and thus parasites, compared to juveniles.

Sol et al. (2003) evaluated these hypotheses using data from a study of feral rock pigeons *(Columba livia)* infected with malaria parasites *(Haemoproteus columbae)* vectored by pigeon louse flies *(*Hippoboscoidea: *Pseudolynchia canariensis).* The authors rejected the vector exposure hypothesis because they found that adult pigeons (>6 months old) are not, in fact, exposed to fewer vectors than juvenile pigeons (Sol et al. 2000). Although the authors reported higher rates of juvenile mortality (61 %) compared to adult mortality (33 %), consistent with the selection hypothesis, selection in their study was not strong enough to explain the lower number of parasites observed in adult birds. The youngest birds in Sol et al.'s study had already fledged from the nest; however, the greatest impact of *H. columbae* on pigeons may occur while birds are still in the nest. We conducted a study to test the impact of *H. columbae* on the condition and fledging success of younger, nestling rock pigeons. We used an experimental approach in which we compared nestlings injected with *H. columbae* to control birds not injected with the parasite.

At least 200 species of *Haemoproteus* are known to infect birds worldwide (Martinsen et al. 2008). Perez-Tris et al. (2005) classified *Haemoproteus* as an avian malaria parasite because members of the genus were nested phylogenetically within the genus *Plasmodium*. *H. columbae* is a parasite of pigeons and doves that uses blood-feeding pigeon flies as vectors (Valkiūnas 2005). The parasite enters a feeding fly and reproduces in its midgut, where *H. columbae* oocysts attach to the gut wall. Once mature, the oocysts burst and release infective sporozoites that migrate from the fly's gut into its salivary glands. The fly then injects these sporozoites into a pigeon when it feeds. *H. columbae* reproduces asexually in the lungs of the pigeon, then invades and matures in the red blood cells (Ahmed and Mohammed 1978).

Haemoproteus species can have several negative effects on host fitness. These effects include reductions in host body condition (Merino et al. 2000), lower reproductive success (Marzal et al. 2004; Tomas et al. 2007), and even death (Atkinson and Forrester 1988; Sol et al. 2003). Studies of the impact of malaria on juvenile birds have consisted of observational studies in the field (Sol et al. 2003), and experimental studies using captive birds (Yorinks and Atkinson 2000; Garvin et al. 2003). The goal of our study was to use an experimental approach under field conditions. We infected nestling birds with malaria parasites to test the impact on body mass, fledging success, age at fledging, and post-fledging survival of experimental versus control birds. Studies with captive birds suggest that the most pathogenic phase of the *Haemoproteus* life cycle occurs when parasites enter red blood cells to mature (Atkinson and Forrester 1988; Atkinson and van Riper 1991). In the case of *H. columbae* this takes place about 24–37 days after infection (Ahmed and Mohammed 1978). Since pigeons fledge at about 32 days of age, it is not possible to be sure that fledglings are infected with malaria parasites, short of experimentally infecting them. Experimental manipulation is the most powerful approach for testing the impact of parasites on hosts in any case (McCallum and Dobson 1995). To our knowledge, this is the first study to test the impact of avian malaria parasites using experimental inoculation under natural conditions.

Materials and methods

We experimentally manipulated *H. columbae* in nestling rock pigeons. The study took place August–November 2009 under a highway overpass in Draper, Utah, USA (40°31′36″N, 111°53′28″W). We visited the field site every 2–3 days throughout the study period. Nestlings were weighed at each visit to the nearest 1.0 g with a pesola scale. Our experiment was restricted to nests with two nestlings, the normal number for rock pigeons. Nests were sequentially assigned to one of three treatment groups: experimental (n = 12 nests), control (n = 13), or background (n = 12). When nestlings were 4–7 days old (50–150 g), those at experimental nests were injected with a suspension of *P. canariensis* flies infected with *H. columbae* (Ahmed and Mohammed 1978). We created the infected fly suspension by feeding flies (bred from wild stock) on heavily infected captive birds. Following 10–12 days on a bird, flies were placed in vials and taken to the field site, where batches of ten live flies were macerated in 1,000 μL of phosphate buffered saline for 3 min. Experimental nestlings were injected intraperitoneally with 500 μL of the infected fly suspension using a 0.5 cc syringe. Control birds were injected with 500 μL of another suspension made using uninfected flies. Background birds were handled but not injected.

Prior to the field experiment, we conducted a test of the inoculation method using 27 wild trapped, captive rock pigeons. After blocking by capture date and site, 13 randomly chosen birds were injected with a suspension of infected flies, as described above. Fourteen control birds were injected with a suspension of uninfected flies. At 25, 35, and 42 days post injection, blood samples were taken from all birds and smears were prepared for examination. Each smear was carefully examined under oil immersion at 1,000 × for 10 min; if parasites were detected, then the number of parasites was quantified in 25 microscope fields per bird. All 13 experimental birds were infected with *H. columbae*, while none of the 14 control birds was infected.

When nestlings were approximately 10 days old they were fitted with a numbered aluminum band and three plastic color bands. To score fledging success we observed and identified birds after they left the nest on the basis of their color band combinations. We conducted a thorough census of all birds at the bridge during each visit to the field site. We also searched for banded birds at other bridges within 8 km of the study site in order to determine whether newly fledged birds were dispersing from the natal site.

We continued to monitor birds at the bridge for 50 days post injection (ca. 25 days post fledging) because peak parasitemia can be delayed for this long after injection (extrapolated from Ahmed and Mohammed 1978). To confirm experimental infections, we examined the blood of birds after they fledged. We used walk-in traps to capture pigeons from 30–50 days post injection. Blood samples were taken and birds immediately released. Blood smears were prepared and examined back in the lab.

Data were analyzed using Prism® v.5.0b (GraphPad Software, Inc.). Power analyses were conducted in G*Power 3 with an error probability set at 0.05 (Buchner et al. 1997). Where necessary, data were log transformed for normalization. To avoid pseudoreplication (Hurlbert 1984) we averaged values for nestlings within each nest. We used one-way ANOVAs to compare parasite abundance and host age and mass at fledging among treatments. A repeated-measures ANOVA was used to compare the number of birds per nest at hatching, fledging, and 1, 2, and 3 weeks post-fledging.

Results

Three times as many experimental birds were infected as control or background birds (Fig. 1a); the three groups also differed in parasite abundance (Fig. 1b; ANOVA $F_{2,17} = 4.25$, $P < 0.05$). Dunnett's post hoc comparisons confirmed that experimental birds had significantly more parasites than controls ($P < 0.05$), while control and background birds did not differ significantly ($P > 0.05$).

There was no significant difference in the age of birds at fledging, nor body mass prior to fledging (see Table 1). There was no significant difference in the proportion of nests that fledged at least one offspring ($\chi^2 = 0.005$, $P = 0.99$). There was also no significant effect of treatment on the mean number of birds fledged per nest, nor the number of birds observed after fledging (Fig. 2; repeated measures ANOVA, treatment $F_{2,34} = 0.64$, $P = 0.53$). There was a significant effect of time (Fig. 2; time, $F_{4,136} = 43.32$, $P < 0.0001$), but no significant interaction between time and treatment (time*treatment, $F_{8,136} = 0.49$, $P = 0.86$).

We reanalyzed the data after excluding naturally infected control and background birds, as well as experimental birds for which we could not confirm infection. We still found no significant difference in age at fledging ($F_{2,31} = 0.53$, $P = 0.60$) mass at fledging ($F_{2,31} = 1.01$, $P = 0.38$), or the proportion of nests that fledged at least one offspring ($\chi^2 = 0.01$, $P = 0.99$).

Our experiment had considerable power (1.0) to detect the level of juvenile mortality (61 %) reported by Sol et al. (2003), we had power of 0.8 to detect mortality of at least 30 % (effect size of f = 0.55).

Discussion

Our goal was to experimentally test the "selection" hypothesis. This hypothesis, reviewed by Gregory et al. (1992), states that lower parasite loads of adults, compared to juveniles, are the result of heavily infected juveniles dying before adulthood, removing parasites from the population. Previous tests of this hypothesis involving avian malaria have focused on juvenile (fledged) birds and relied on observational data (Sol et al. 2003;

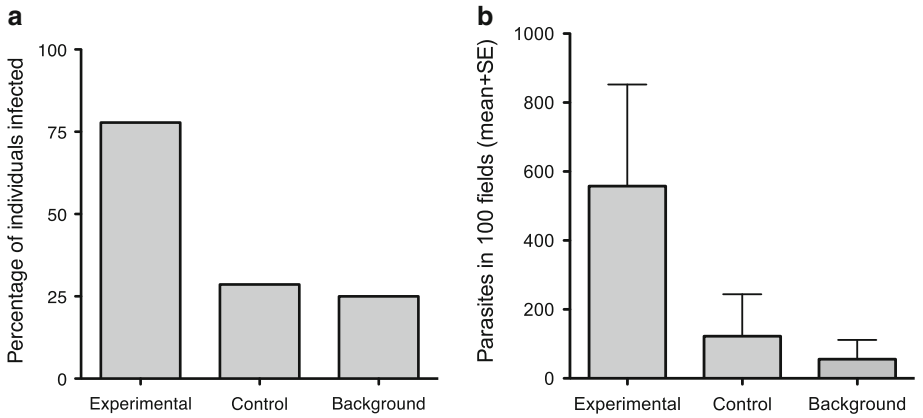

Fig. 1 Prevalence **(a)** and mean abundance (+SE) **(b)** of malaria parasites 30–50 days after treatment

Table 1 Age of birds at fledging and body mass prior to fledging. Values are grand means (±SE) of the mean value per nest

	Experimental	Control	Background	Test statistic	*P*
Age in days	32.3 ± 0.5	31.8 ± 0.6	32.3 ± 0.6	$F = 0.25$	0.78
(Number of nests)	(11)	(12)	(11)		
Mass in grams	298 ± 14.9	311.3 ± 12.5	313.1 ± 11.4	$F = 0.35$	0.71
(Number of nests)	(11)	(12)	(11)		

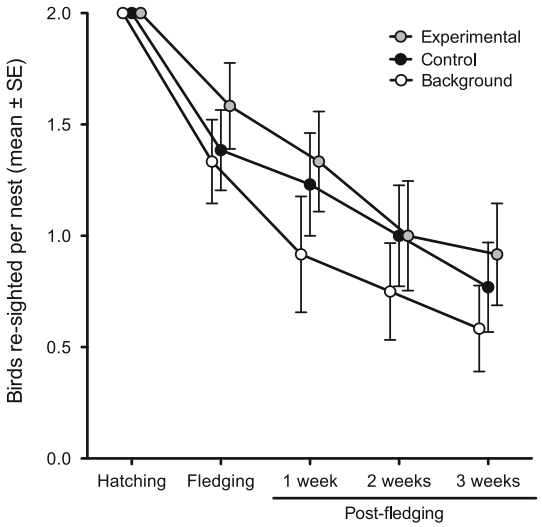

Fig. 2 Mean (±SE) offspring observed per nest. The mean (±SE) number of offspring fledged per nest did not differ significantly among treatments. Points are slightly offset for clarity

van Oers et al. 2010). These studies provided some support for the selection hypothesis, but the intensity of selection measured could not fully explain differences in juvenile and adult parasite loads. It was conceivable, therefore, that the greatest impact of *H. columbae* on pigeons takes place while they are still in the nest.

Our results provided no support for the selection hypothesis because there was no impact of malaria on any of the components of host fitness we measured. Specifically, there was no significant difference in the body mass, fledging success, age at fledging, or post-fledging survival of experimental versus control birds. We are confident that our measures of post-fledging survival were accurate because none of the birds from our study were observed at other bridges (see methods). Young pigeons do not normally disperse until 3 months of age, in any case (Johnston and Janiga 1995).

The results of our study were unexpected, given that Sol et al.'s longer-term study demonstrated that *H. columbae* has a significant negative impact on pigeon fitness. The fact that malaria had no detectable impact on fledging success in our study was not due to unusually low rates of fledging in both experimental and control birds. Fledging success was 73 % (Fig. 2), similar to that in other studies of feral pigeons [reviewed by Johnston and Janiga (1995), Table 18.4 (values adjusted for hatching rates)]. Similarly, the fact that malaria had no detectable impact on fledging was not due to methodological problems with the creation of experimental infections. The malaria parasite levels in our study were comparable to those observed in other studies of naturally infected pigeons (Kartman 1949;

Klei and DeGuisti 1975; Paperna and Smallridge 2002). However, *H. columbae* may affect hosts only at levels higher than what we observed (Earle et al. 1993; Paperna and Smallridge 2002). For example, the *H. columbae* levels in Sol et al.'s (2003) study were among the highest ever recorded for feral rock pigeons.

Another factor that could conceivably contribute to why the birds in our study did not appear to be affected by *H. columbae*, compared to the reduction in survival shown for older birds by Sol et al. (2003), is that nestling pigeons could have higher tolerance to parasites than older birds. Nestlings are fed a rich diet of crop milk by both parents. The milk, which consists of the sloughed lining of the parents' crop, is very high in fat and protein (Johnston and Janiga 1995). It would be interesting to test the impact of *H. columbae* on nestlings fed a less nutritious diet.

A few control and background birds were naturally infected with *H. columbae*. However, infection levels were still significantly higher in the experimental group than the control or background groups. Even after excluding the naturally infected birds, we did not find that malaria parasites affected fledging age or mass, or fledging success.

Since *H. columbae* had no apparent effect on nestling rock pigeons, our study does not provide support for the "selection hypothesis". Sol et al. (2003) reported results that were consistent with selection hypothesis; however, selection in their study was not strong enough to explain the differences in parasitemia they observed between juvenile and adult pigeons. Because Sol et al. (2000, 2003) reported data ruling out the "vector exposure" hypothesis, they suggested a combination of the selection and immunity hypotheses may explain the fact that juvenile birds have higher parasitemia than adult birds. Our data provide no reason to disagree with this assessment.

To our knowledge, this is the first study to test the impact of avian malaria parasites using experimental inoculation under natural conditions. This approach has several advantages. First, like many malaria parasites, *H. columbae* takes several weeks to appear in the peripheral blood after the host is infected. This fact makes early infections difficult to detect without more invasive methods, such as collection of organ tissues (Valkiūnas 2005; Cosgrove et al. 2006). Experimental infections get around this problem. Second, inoculating hosts with parasites has the strong advantage of controlling for factors that could lead to spurious negative correlations between parasite load and host fitness (Hawlena et al. 2006; Blanchet et al. 2009). The greatest limitation of our study is that the modest sample sizes limit our ability to detect relatively small effect of malaria parasites on birds. For example, to detect a 10 % reduction in juvenile survival with a power of 0.8 would require a sample of 93 nests per treatment for a total of 279 nests. A study of this magnitude may be feasible in the future using feral Rock Pigeons and *H. columbae.*

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