

# The blood parasite *Haemoproteus* reduces survival in a wild bird: a medication experiment

Josué Martínez-de la Puente<sup>1,\*</sup>, Santiago Merino<sup>1</sup>, Gustavo Tomás<sup>1,2</sup>, Juan Moreno<sup>1</sup>, Judith Morales<sup>1,3</sup>, Elisa Lobato<sup>1,4</sup>, Sonia García-Fraile<sup>1</sup> and Eduardo Jorge Belda<sup>5</sup>

<sup>1</sup>Departamento Ecología Evolutiva, M.N.C.N.-C.S.I.C., C/ José Gutiérrez Abascal 2, 8006 Madrid, Spain

<sup>2</sup>Departamento Ecología Funcional y Evolutiva, Estación Experimental de Zonas Áridas (C.S.I.C.), Carretera de Sacramento s/n, La Cañada de San Urbano, 04120 Almería, Spain

<sup>3</sup>Departamento Ecología Animal, Facultad de Biología, U.V., 36310 Vigo, Spain

<sup>4</sup>Centre d'Ecologie Fonctionnelle et Evolutive CNRS, 1919 Route de Mendre, Montpellier, France

<sup>5</sup>Instituto de Investigación para la Gestión Integrada de Zonas Costeras-IGIC, U.P.V., Carretera Nazaret-Oliva s/n, 46730 Gandia, Valencia, Spain

\*Author for correspondence (jmp@mncn.csic.es).

**While avian chronic haemoparasite infections induce reproductive costs, infection has not previously been shown to affect survival. Here, we experimentally reduced, through medication, the intensity of infection by *Haemoproteus* parasites in wild-breeding female blue tits *Cyanistes caeruleus*. However, this treatment did not reduce the intensity of infection in males or the intensity of infection by *Leucocytozoon*. Medicated females, but not males, showed increased local survival until the next breeding season compared with control birds. To our knowledge, this is the first empirical evidence showing long-term direct survival costs of chronic *Haemoproteus* infections in wild birds.**

**Keywords:** host–parasite interactions; parasitism costs; sexual differences

## 1. INTRODUCTION

Parasitism is a major selection force affecting wild animals. The phylum Apicomplexa forms a large and cosmopolitan assemblage of protozoan parasites. Within this phylum are the haemosporidians, including malaria parasites, which infect blood-feeding dipterans acting as vectors, and several classes of vertebrates. These parasites are common infections in many bird species and there are evidences for short-term costs of chronic infections by the haemosporidians parasites *Plasmodium*, *Haemoproteus* and *Leucocytozoon* on wild bird's reproductive performance (e.g. Merino *et al.* 2000; Marzal *et al.* 2005; Knowles *et al.* 2010). However, effects of these parasites on survival have remained elusive in wild avian populations (e.g. Stjernman *et al.* 2004). Thus, it has been unclear

whether infections can have long-term fitness consequences, which is an important assumption of many studies of host–parasite interactions.

Intraspecific (Richner *et al.* 1995; Nordling *et al.* 1998; Marzal *et al.* 2008) and comparative studies (Møller & Nielsen 2007) suggest a role of blood parasites in reducing avian survival under natural conditions. However, these studies have not controlled for differences in capture probability, making previous conclusions open to alternative interpretations. In addition, no study has experimentally investigated host survival in relation to infections by *Haemoproteus* parasites in wild populations, probably owing to the difficulty of manipulating parasite loads. The use of the antimalarial drug primaquine has overcome this challenge allowing experimental reductions of *Haemoproteus* loads in birds and investigation of subsequent effects on post-breeding body condition (e.g. Merino *et al.* 2000) or reproduction (e.g. Merino *et al.* 2000; Marzal *et al.* 2005).

We tested whether experimental reduction in the intensity of infection by *Haemoproteus* parasites increases local survival in its avian host. Owing to the effect of host sex on parasite load and the efficacy of antiparasitic treatments (e.g. Klein 2004; Martínez-de la Puente *et al.* 2007), we also explored a potential differential effect of medication on parasite load and subsequent survival between sexes. Because treatment causes detectable reductions in *Haemoproteus* load in females, but not in males (Martínez-de la Puente *et al.* 2007), we predicted an increase in female, but not in male survival following medication.

## 2. MATERIAL AND METHODS

This study was conducted on blue tits (*Cyanistes caeruleus*) breeding in nest boxes in Spain (40°53' N, 4°01' W, 1200 m above sea level). During 2004, birds were captured and blood sampled when their nestlings were 3 days old (initial sample). Birds attending nests with similar clutch size ( $\pm 1$  egg) and hatching date ( $\pm 1$  day) were randomly assigned to one of the two treatments (medicated or control). Previous studies revealed that the prevalence of infection by *Haemoproteus* spp. and *Leucocytozoon* spp. in our population is very high, thus allowing blind assignment of treatments. This assumption was later confirmed (see § 3). Medicated birds were injected subcutaneously with 0.1 mg primaquine (Sigma, St Louis, MO, USA) diluted in 0.1 ml saline solution, whereas control birds were injected with saline solution only (Merino *et al.* 2000). Ten days later birds were recaptured and a second blood sample obtained (final sample). All birds breeding in nest boxes in the study area were captured each season until 2007, allowing analysis of local survival in relation to treatment.

A drop of blood from each sample was immediately smeared, air-dried, fixed in absolute ethanol and stained with Giemsa for 45 min. Smears were used to quantify intensity of infection by *Haemoproteus* spp. and *Leucocytozoon* spp. (see electronic supplementary material). An additional drop of blood was collected in a plastic tube and frozen for molecular detection of parasite infection. Blood samples from birds diagnosed as uninfected by inspection of smears at the initial capture were also analysed with polymerase chain reaction (see electronic supplementary material). Blood samples from one female and five males were insufficient for conducting DNA analyses.

We used repeated-measures ANOVA to test for an effect of treatment on change in intensity of infection by *Haemoproteus* and *Leucocytozoon* parasites between initial and final samples in the two sexes separately. Intensities of infection were log-transformed to allow use of parametric statistics. Survival analyses were carried out using two different methods. First, for simplicity, we tested for an effect of treatment and sex (as factors) on local survival using a generalized linear model (GLM) model. The main problem with this approach is that it does not consider either capture probability or permanent emigration. Therefore, we also estimated survival using capture–mark–recapture models for open populations (see electronic supplementary material).

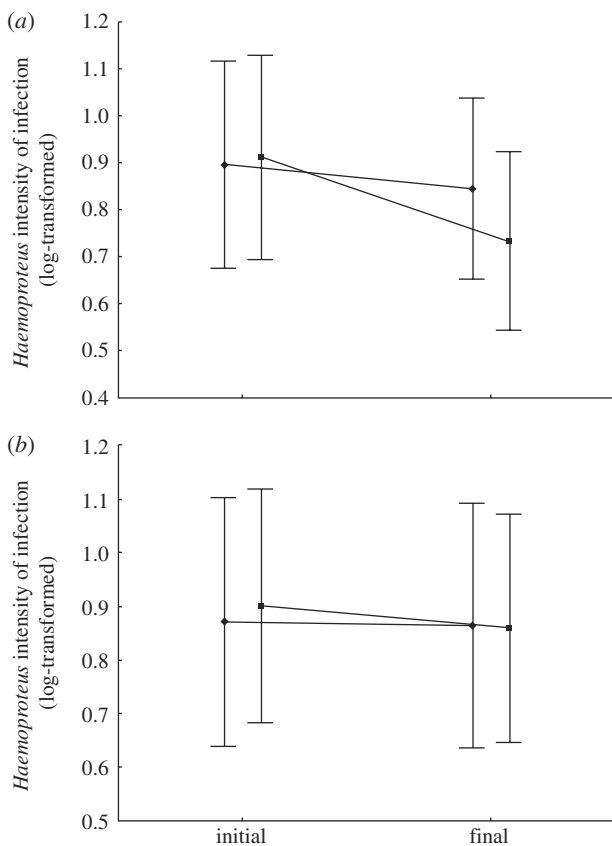


Figure 1. Change in the intensity of infection by *Haemoproteus* parasites (log-transformed) in (a) female and (b) male blue tits with respect to the medication treatment (females:  $F_{1,77} = 4.25$ ,  $p = 0.04$ ; males:  $F_{1,73} = 0.34$ ,  $p = 0.56$ ). Bars denote 95% confidence intervals. Solid line connecting filled diamonds, control; dashed line connecting squares, medicated.

### 3. RESULTS

A total of 95 females (47 medicated, 48 controls) and 92 males (47 medicated, 45 controls) were included in the experiment. At first capture, 85 per cent of females and 88 per cent of males were infected with *Haemoproteus* spp. (see also table S1 in the electronic supplementary material), whereas 92 per cent of females and 87 per cent of males were infected by *Leucocytozoon* spp. All except one male and one female were infected by at least one parasite.

Repeated-measures ANOVA revealed that medicated females experienced a significantly greater reduction in intensity of infection by *Haemoproteus* parasites from initial to final samples than control females (figure 1a;  $F_{1,77} = 4.25$ ,  $p = 0.04$ ). In males, change in intensity was not significantly affected by treatment (figure 1b;  $F_{1,73} = 0.34$ ,  $p = 0.56$ ). There were no significant treatment effects on change in intensity of infection by *Leucocytozoon* parasites in either sex (all  $p > 0.35$ ).

Of all birds included in the study in 2004, 28 females (19 medicated and nine controls) and 26 males (11 medicated and 15 controls) were recaptured at least once between 2005 and 2007. Sex and treatment were not significantly associated with local survival, but there was a significant interaction effect on survival (GLZ: treatment: Wald = 0.77,  $p = 0.38$ ;

Table 1. Survival probabilities  $\pm$  standard error (s.e.) and 95% confidence interval for control and medicated blue tits. (Estimation was done using model averaging.)

parameter	survival probability	s.e.	95% confidence interval	
			lower	upper
control female	0.22	0.08	0.12	0.42
medicated female	0.42	0.09	0.24	0.60
control male	0.39	0.07	0.23	0.53
medicated male	0.27	0.06	0.18	0.46

sex: Wald = 0.001,  $p = 0.97$ ; treatment  $\times$  sex interaction: Wald = 5.56,  $p = 0.02$ ), supporting an effect of medication on survival in females only. Accordingly, the best model for survival included an interaction between sex and treatment in the first year post-treatment (model 1, table S2 in the electronic supplementary material). The other three competing models included within two Akaike Information Criterion with second order correction units of the best model (table S2 in the electronic supplementary material) support the interaction between sex and treatment on survival between 2004 and 2005, although differing in effect of treatment on survival to subsequent years. Furthermore, the only partial coefficient of the log-linear function for survival for the best-fit model with a 95% confidence interval that did not overlap with zero included the interaction between sex and treatment (Beta (s.e.) = 1.71 (0.60), 95% confidence interval = 0.53–2.89). This indicates that survival probability of treated females was higher than for controls, and there was no significant effect of primaquine treatment on male survival. We estimated survival probabilities for males and females assigned to different treatments by model averaging, and the higher survival probability was obtained for medicated females (table 1). For additional information, see the electronic supplementary material.

### 4. DISCUSSION

There was a sex-specific effect of medication on *Haemoproteus* parasite intensity. Host sex is an important determinant of intensity of infection by parasites (Møller *et al.* 1998), with males usually having stronger infections (Klein 2004). Although reports on the pharmacokinetics of treatments against parasites in wild animals of different sexes are scarce, the literature reports sex effects on drug kinetics (e.g. Klein 2004; Dimitrova *et al.* 2009), including studies with antimalarial drugs (Gordi *et al.* 2002). Different factors tightly related to sex, such as hormone concentrations and genetic characteristics, may affect the absorption and metabolism of drugs (Lashev *et al.* 1995; Pinsonneault & Sadée 2004). These factors may be implicated in the observed different efficacy of primaquine treatment between sexes.

Experimental reductions of helminth loads increase adult survival in red grouse *Lagopus lagopus* (Hudson & Dobson 1991) and common eiders *Somateria mollissima* (Hanssen *et al.* 2003). By direct experimental manipulation of intensity of infection of a common avian parasite through medication, we have, to our knowledge, shown for the first time that chronic *Haemoproteus* parasite infections have detrimental effects on survival. In accord with expectations, female but not male blue tits increased survival following medication, consistent with the fact that females were the only sex in which medication effectively reduced the intensity of infection. Reductions of parasite loads may imply beneficial effects for hosts in terms of reduction of adverse effects of parasitism, including the amount of resources drained by the parasite and the amount of resources devoted by hosts to immune defence (de Lope *et al.* 1998; Martínez *et al.* 2004). In addition, both infection status and immunological response of hosts may increase metabolic rate (Martínez *et al.* 2004) and, as a consequence, reduce survival by increasing susceptibility to infection by other pathogens or capture by predators (Hudson *et al.* 1992; Møller & Nielsen 2007). Our findings add to previous studies showing short-term costs of *Haemoproteus* parasite infections on avian post-breeding body condition (Merino *et al.* 2000) and reproductive success (Merino *et al.* 2000; Marzal *et al.* 2005).

The study complies with current laws in Spain.

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