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Malarial parasites decrease reproductive success: an experimental study in a passerine bird

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Abstract Malarial parasites are supposed to have strong negative fitness consequences for their hosts, but relatively little evidence supports this claim due to the difficulty of experimentally testing this. We experimentally reduced levels of infection with the blood parasite *Haemoproteus prognei* in its host the house martin *Delichon urbica*, by randomly treating adults with primaquine or a control treatment. Treated birds had significantly fewer parasites than controls. The primaquine treatment increased clutch size by 18%; hatching was 39% higher and fledging 42% higher. There were no effects of treatment on quality of offspring, measured in terms of tarsus length, body mass, haematocrit or T-cell-mediated immune response. These findings demonstrate that malarial parasites can have dramatic effects on clutch size and other demographic variables, potentially influencing the evolution of clutch size, but also the population dynamics of heavily infected populations of birds.

Keywords Blood parasites · *Delichon urbica* · Haematozoa · Primaquine · Reproductive success

Introduction

Parasites are ubiquitous and have drastic effects on their hosts due to their exploitation of resources that could otherwise be used by the hosts (Noble and Noble 1976; Price 1980; Combes 2001). It is thus not surprising that most aspects of the life history of hosts such as age when reaching maturity, clutch size and offspring size are believed to be affected by parasites (Hochberg et al. 1992; Lehmann 1993; Møller 1997). Results consistent with these ideas have begun to emerge during the last decades but our knowledge of causal relationships is still rudimentary due to a scarcity of experimental manipulation.

Malaria is a major cause of death in humans (Miller et al. 2002), but presumably also in many other organisms (Atkinson and Van Riper 1991; Bennett et al. 1993). Malaria is supposed to have strong negative effects on host fitness because the group of intra-cellular parasites involved causes dramatic effects on the efficiency of metabolism (Chen et al. 2001). Malarial parasites are also some of the most widespread parasites, with prevalence often reaching 100%, and infections being chronic (Atkinson and Van Riper 1991). However, the fitness consequences of malarial infections are poorly known. Several studies have shown that an experimental increase in parental investment causes an increase in levels of infection (Oppliger et al. 1996; Merino et al. 1996; Allander 1997; Merilä and Andersson 1999; Wiehn et al. 1999). Likewise several studies have suggested that malarial parasites lead to a reduction in a range of different fitness components, but the results are mixed in terms of magnitude of effect (Korpimäki et al. 1995; Sundberg 1995; Oppliger et al. 1997; Bennett et al. 1988; Dawson and Bortolotti 2000; Sanz et al. 2001). Most studies have revealed weak effects explaining at most a few percent of the variance. The main problem with all these studies is the lack of experimentation. So far only a single study has experimentally treated malarial infection in birds. Merino et al. (2000) treated blue tits *Parus caeruleus* with primaquine after the start of laying, causing a significant decrease in the level of infection and an increase in reproductive success. Thus, there is evidence of a direct effect of avian malaria on reproductive output.

The objective of this study was to investigate how malaria infection affected clutch size, using an experimental manipulation of infection status with the antimalarial agent, primaquine. Experiments of this type are

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important because they can be used to assess whether effects of malarial infection early during the reproductive cycle have disproportionately large effects on seasonal reproductive success. This requires treatment of adults well before the start of reproduction. At the start of reproduction and the production of sex hormones, individuals with chronic infections of malarial parasites show dramatic relapses (e.g. Chernin 1952; Applegate and Beaudoin 1970; Allander and Sundberg 1997). Thus early treatment of individual hosts is expected to reduce or maintain levels of infection at a time in the reproductive cycle when infections are normally increasing rapidly. We experimentally treated house martins *Delichon urbica*, a colonial passerine migratory bird with high levels of prevalence of the parasite *Haemoproteus prognei*.

Materials and methods

In March–May 2002, 49 pairs of house martins were chosen before they had laid eggs and were randomly assigned to one of two treatments. Treatment occurred on average 9.37 days (SD = 1.97) before egg laying started. We treated 52 adults from 26 nests while keeping 46 adults from 23 nests as controls. Poor weather caused considerable levels of desertion that resulted in 32 treated adults and 28 controls being assessed for effects of treatment on reproduction. There were no significant differences in capture date (Mann–Whitney *U*-test, $z = -0.18$, $P = 0.87$), body mass (Mann–Whitney *U*-test, $z = -0.44$, $P = 0.68$), wing length (Mann–Whitney *U*-test, $z = -1.27$, $P = 0.22$), tarsus length (Mann–Whitney *U*-test, $z = -0.45$, $P = 0.65$) and haematrocrit (Mann–Whitney *U*-test, $z = -0.19$, $P = 0.85$) between treated individuals and controls among the birds that had deserted. Since only half of all pairs (56%) lay second clutches (Pajuelo et al. 1992), only data from first clutches were recorded.

Individuals were captured at dawn in their nest and injected sub-cutaneously with either 0.01 mg primaquine (Sigma, St. Louis, Mo.) in 0.1 ml saline solution or the same quantity of pure saline solution (controls), following the procedure reported by Merino et al. (2000). Primaquine is an anti-malarial chemical compound used in different treatments to reduce the level of parasitaemia in birds (Redig et al. 1993). It results in dose-dependent effects, such as gastrointestinal disturbances and development of met-haemoglobinaemia and haemolytic anaemia (Mayorga et al. 1997). Thus, in order to minimize these effects we reduced treatment to a low-concentration single dose. In any case, the toxicity of primaquine rules out the possibility of beneficial side-effects of medication, other than a reduction in blood parasitism (see Merino et al. 2000).

Blood samples for haematological measurements were taken from the brachial vein immediately before treatment. For identification of blood parasites, a drop of blood was smeared on a microscope slide and air-dried. All birds were individually identified with numbered metal rings.

Nests were inspected every day to obtain information on laying date, clutch size, hatching date and brood size (day 1 = 10 March).

When nestlings were 12 days old, we recaptured parents and took a second blood sample to check for the effect of treatment on parasite intensities and prevalence.

Blood samples were fixed in absolute methanol and stained with Giemsa. Half of each smear was examined under 200× magnification in search of large extra-erythrocytic Haematozoa (i.e. *Trypanosoma*), and in the other half of the smear 20 fields were scanned under 400x magnification for intra-erythrocytic Haematozoa (i.e. *Haemoproteus*). The intensity of extra-erythrocytic parasites was quantified as number of parasites per 100 fields, and number of parasites per 2,000 erythrocytes for intra-erythrocytic parasites under 1,000× magnification with oil immersion (Merino et al. 1997).

As a measure of immune response, we used the T-cell-mediated immune response to a challenge with phytohaemagglutinin (PHA). This is a standard estimate from the poultry literature of the ability to produce a T-cell-mediated immune response (Goto et al. 1978; Parmentier et al. 1993). Injection with PHA results in local activation and proliferation of T-cells, followed by local recruitment of inflammatory cells and increased expression of major histocompatibility complex molecules (Goto et al. 1978; Abbas et al. 1994).

Fifteen-day-old nestlings were injected with 0.05 ml of 0.2 mg PHA-P in one wing web (Smits et al. 1999). All individuals were injected in the afternoon between 1600 and 1800 hours. The dose of PHA used in this study is similar to that used in numerous other studies of free-living or captive birds (Saino et al. 1997; Christe et al. 1998, 2000; Birkhead et al. 1999; González et al. 1999; Hõrak et al. 1999; Soler et al. 1999; Merino et al. 2000; Navarro et al. 2003). We measured the thickness of the patagium (an expandable membranous fold of skin between the wing and body of a bird) injected with PHA after 24 h, using a pressure-sensitive spessimeter (Digimatic Indicator ID-C, Mitutoyo Absolute 547-301, Japan) with an accuracy of 0.01 mm. In the subsequent analyses we used the increase in wing thickness as a measure of the intensity of the PHA-induced immune response (hereafter T-cell response). On the first capture, we measured body mass with a Pesola spring balance to the nearest 0.1 g, and tarsus length with a digital calliper to the nearest 0.01 mm. Micro-capillary tubes were centrifuged for 10 min at 14,000 r.p.m. to estimate haematocrit (the proportion of blood volume occupied by red blood cells).

Results

At the start of the breeding season, 48.3% of adults ($n = 60$) were infected with *Haemoproteus*, the only

common parasite in the population of house martins. Before treatment with primaquine no significant difference in prevalence ($\chi_i^2 = 0.63$, $P = 0.43$) or intensity of parasitism between groups was observed (Mann–Whitney U-test, $z = -1.33$, $P = 0.18$; 32 treated and 28 controls). As expected from the anti-malarial treatment, there was a significant decrease in prevalence (McNemar $\chi^2 = 3.77$, $P = 0.049$; Fig. 1a) and in intensity of parasitism (Wilcoxon matched-pairs signed-ranks test, $z = -3.29$, $P = 0.001$; Fig. 1b), while for controls there was a significant increase in the proportion of individuals that became infected (McNemar $\chi^2 = 8.64$, $P = 0.002$; Fig. 1a), and in the intensity of parasitism (Wilcoxon matched-pairs signed-ranks test, $z = -2.43$, $P = 0.015$; Fig. 1b). In addition, more individuals remained uninfected in the treated group (34.4%) than among controls (10.7%) ($\chi^2 = 9.314$, $P = 0.002$).

Clutch size, brood size at hatching and brood size at fledging all differed significantly between groups (Table 1). Clutch size was on average 18% larger in treated birds, while the difference increased to 39% at hatching and 42% at fledging. Hatching success was significantly greater in the treated birds, suggesting that egg quality and/or incubation behaviour was affected by the treatment (Table 1). In contrast, there was no significant difference for fledging success or overall breeding success (Table 1). The difference in clutch size between treatment group was small and did not reach significance, using final *Haemoproteus* infection intensity as a covariate in an analysis of covariance ($F_{1,57} = 3.52$, $P = 0.07$).

The quality of offspring measured in terms of body mass, tarsus length, haematocrit or T-cell-mediated immune response did not differ significantly between groups (Table 1).

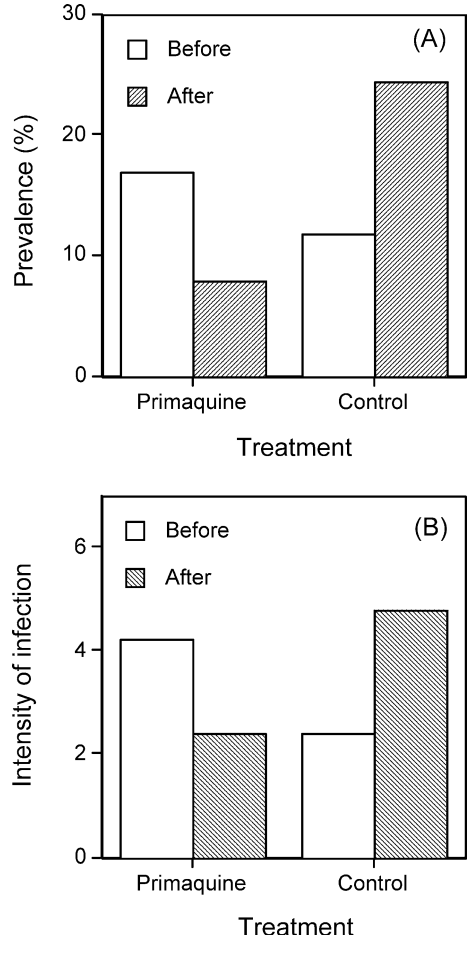

Fig. 1 **a** Prevalence (%) and **b** intensity of infection with *Haemoproteus prognei* in adult house martins before and after treatment with primaquine. Sample sizes are 16 treated pairs and 14 untreated controls

Discussion

Clutch size increased by 18% as a consequence of primaquine treatment (Table 1). Numerous hypotheses have been put forward to explain optimal clutch size (review in Roff 2001). The importance of parasitism for clutch size evolution has not usually been appreciated, and only very few studies have addressed this question despite the ubiquitous presence of parasites in all hosts (Møller 1991; Richner and Heeb 1995; Martin et al. 2001). One of the hypotheses addressing the evolution of optimal clutch size relates directly to the impact of parasites on host reproductive success, when individuals within a large clutch suffer disproportionately from the negative effects of parasitism (Møller 1991). Such an effect occurs when parasite populations grow particularly rapidly in large broods of hosts, or when the fitness consequences of parasitism are particularly severe in large broods, because more nestlings are negatively affected by parasitism (Møller 1991). Our present study is the first to demonstrate that there is indeed an improvement in clutch size as a response to a reduction in the number of parasites. We can only speculate about possible mechanisms generating this effect because we did not assess them directly. Firstly, malarial parasites may directly impact foraging ability and therefore rate of level of resource acquisition necessary for production of eggs. Secondly, components used by the immune system for fighting serious infections may also play a role in egg formation. Blount et al. (2004) have recently shown that carotenoid availability limits egg production in birds, and Saino et al. (2003) have shown that carotenoids limit the T-cell-mediated immune response implicated in anti-malarial immune defence (Wakelin 1996). Finally, by definition, parasites drain energy from their hosts because they extract nutrients that could otherwise have been used by the host (Price 1980), and immune function requires resources that might otherwise have been used for other functions (Sheldon and Verhulst 1996).

While the difference in clutch size between groups was on average 18%, the difference in hatching was 39%, due to a significant effect of treatment on hatching success, and the difference in fledging was 42% (Table 1). The effect of treatment on hatching success

Table 1 Mean (SD) reproductive success and nestling quality of treated ($n = 16$) and control nests ($n = 14$). The number of nestlings in treated nests was 45 and in controls 22. Differences for nestlings were tested by using brood means

	Treatment	Control	z (Mann–Whitney U-test)	P
Clutch size	4.44 (0.62)	3.64 (0.93)	−2.466	0.02
Brood size at hatching	3.37 (1.25)	2.07 (0.83)	−3.352	0.001
Brood size at fledging	2.81 (1.11)	1.64 (0.63)	−3.350	0.001
Hatching success	0.75 (0.27)	0.58 (0.26)	−2.233	0.03
Fledging success	0.85 (0.16)	0.82 (0.20)	−0.496	0.65
Breeding success	0.63 (0.26)	0.48 (0.27)	−1.842	0.07
Haematocrit (%)	45.52 (2.76)	46.53 (3.18)	−0.831	0.41
Body mass (g)	17.69 (1.16)	17.40 (1.15)	−0.577	0.59
Tarsus length (mm)	10.29 (0.32)	10.31 (0.34)	−0.448	0.68
T-cell response (mm)	1.49 (0.43)	1.35 (0.18)	−0.30	0.79

may either be due to an effect of treatment on egg quality or on incubation behaviour. If there is a trade-off between use of carotenoids and other anti-oxidants for egg production and immunity, as suggested above (Blount et al. 2004; Saino et al. 2003), we speculate that control females laid eggs with reduced levels of yolk anti-oxidants. This could directly lead to reduced hatching success (Surai 2003). Alternatively, adult house martins may have been affected by malarial infections, causing a reduction in the efficiency of incubation and provisioning for offspring. The latter hypothesis seems less likely since parental effort during the nestling period is considerably greater than during incubation. We should therefore expect much greater effects on fledging success than on hatching success, while in reality the opposite pattern was observed. We suggest two alternative explanations for this result. First, a month passed from treatment to time of fledging, potentially obscuring any effects of treatment on infection status. Second, parents may adjust their parental effort to brood size (see Sheldon and Verhulst 1996)

While parasites can have strong effects on demographic parameters of their hosts, it is less well known whether this has consequences for population dynamics. We can make preliminary estimates of the effects of malarial parasites on the population size of house martins in Spain and Denmark, using the estimated magnitude of effect from the present study. Annual reproductive success of house martins in Spain is on average 4.3 eggs in the first clutch and 3.7 eggs in the second clutch. These 8.0 eggs give rise to, on average, 6.8 fledglings. *Haemoproteus* parasites have a prevalence of 48% in house martins in Spain. In these 48% of house martins, reproductive success where blood parasites are reduced increases by 42%. Therefore, overall production of fledglings is depressed by 40% in the presence of blood parasites. In Denmark, house martins lay on average 4.08 eggs in the first clutch and 67.7% of the birds lay a second clutch with on average 3.36 eggs (Møller 1974). These eggs give rise to on average 5.87 fledglings. Since the prevalence of *Haemoproteus* in Denmark is 0% ($n = 55$ adults, A. P. Møller unpublished data), there is no suppression of reproductive blood parasites. Thus, malarial parasites depress the annual output of fledglings by 40% in the Spanish population, but do not depress it in the Danish population. Therefore, there are considerable effects of blood parasitism on the size of the post-breeding population of hosts, and this may affect the relative contribution of different populations to the overall population size of hosts across their range due to differences in population productivity.

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