

Haemosporidians in migratory blackcaps (*Sylvia atricapilla*): a comparison between autumn and spring periods of passage

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Resumen

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Received: 29 May 2010

Accepted: 13 October 2010

Published on-line: 27 October 2010

Hemosporidios en currucas capirotadas (Sylvia atricapilla) migratorias: comparativa entre los pasos de otoño y primavera.

La relación y distribución espacial y temporal parásito-hospedador tienen un gran interés para comprender los procesos coevolutivos entre ambos. El objetivo del trabajo fue analizar si varió la prevalencia e intensidad de infección por hemoparásitos en currucas capirotadas (*Sylvia atricapilla*), en un área de descanso en el N de España, en los pasos prenupcial y posnupcial. También se estudió si tuvo efecto sobre la carga de reservas del hospedador. Se examinaron frotis de sangre de 132 individuos. Sólo se detectaron *Haemoproteus* spp. y *Plasmodium* spp., pero no *Trypanosoma* spp., microfilarias o *Leucocytozoon* spp. Se registró una prevalencia del 35%, que no varió entre periodos, ni a lo largo de los mismos, ni por edades, pero sí entre sexos. La prevalencia fue superior en machos (45,5% frente a 22,7%), lo cual podría ser debido a diferencias inmunológicas asociadas al sexo. La intensidad de infección fue, en promedio, baja (2,8 parásitos/2000 eritrocitos). No se registró ningún efecto de la parasitosis sobre la carga de reservas.

Palabras clave: Carga de grasa y reservas, Hemosporidios, migración, Parasitos, Península Ibérica.

Abstract

The spatio-temporal host-parasite relationships have a high interest to understand the coevolutionary processes between parasites and their hosts. Our main aim here was to analyse whether the prevalence and intensity of infection by haemoparasites in migrating blackcaps (*Sylvia atricapilla*), at a stopover site in N Iberia, varied throughout and between migration periods. Moreover, we studied whether the parasitaemia had any effect on fuel load. Blood smears were studied from 132 individuals. Only *Haemoproteus* spp. and *Plasmodium* spp. were detected. *Trypanosoma* spp., microfilaria or *Leucocytozoon* spp. were absent. The prevalence was 35% and it did not differ throughout and between periods or between age classes, but between the sexes. Prevalence was higher among males (45.5% versus 22.7%), suggesting that males were more prone to be infected, which could be due to sex-associated immunologic variations. The mean intensity of infection was low (2.8 parasites/2000 erythrocytes). We did not find any effect of parasitaemia on fuel load.

Key words: Haemosporidians, Migration, Parasites, Fuel load, Iberian Peninsula.

Introduction

The spatio-temporal host-parasite relationships have a high interest to understand the mechanisms underlying the coevolutionary processes between parasites and their hosts (Blanco et al. 2001, Bensch & Akesson 2003). Thus, migration allows parasites to spread over large geographic areas (Pérez-Tris & Bensch 2005, Pérez-Tris et al. 2007). A question to be considered in this scenario is to what extent parasite faunas differ geographically (Scheurerlein & Ricklefs 2004, Valkiūnas 2005), and how, or to what extent such a variation is reflected during migration period, assuming that birds from different regions could migrate differentially, hence they could pass through certain stopover sites at different time (e.g. Lövei 1983, Chernetsov 2004).

Haemoparasites mainly depend on an invertebrate vector (usually sucking insects) to infect their avian hosts (Valkiūnas 2005), so infection rate peaks when density of vectors is highest (Bennett et al. 1974, Garvin & Remsen 1997, Sol et al. 2000, Mendes et al. 2005), during late-spring and summer at northern latitudes. Thus, infection rate can also differ temporally. In autumn, the density of vectors may decrease with time, as they should be expected to become less abundant across the season. In spring, however, the density of vectors may increase with time. Alternatively, it could be also possible that due to the warmer temperatures of the non-breeding quarters in S Europe or Africa compared with northern stopover localities, the intensity of infection may be higher during early spring than late spring.

Since parasites obtain resources from their hosts, they constitute a relevant selection pressure (Ricklefs 1992, Hudson et al. 1998). Thus, it is reasonable to find infected birds in worse body condition than non-infected ones, a fact that has been reported both experimentally (Atkinson et al. 1988, Merino et al. 2000) and using data from wild populations (Valkiūnas 1993). However, such a negative effect on body condition does not seem to be always manifest (e.g. Bennett et al. 1988, Arizaga et al. 2009). Valkiūnas (2005) suggested that these differences could be due to the increasing effect on body condition with increasing densities of parasites.

The blackcap (*Sylvia atricapilla*) is an extended Palaearctic songbird (Berthold & Solonen

1997), with its migratory behaviour varying from totally migratory to sedentary (Shirihai et al. 2001). Iberia is one of the main goal wintering areas for large numbers of migrant European blackcap populations (Cantos 1995), with most of them being found in the Meso- and Thermo-Mediterranean area from S and E Iberia (Tellería et al. 1999). Consequently, N Iberia is a stopover region used by populations from practically the whole breeding range of the species for W Europe (from S France to SW Scandinavia and the British Islands), both during autumn (in the way to their wintering areas) and during spring (to their breeding areas).

Our aim here was to analyse whether the prevalence and intensity of infection by haemoparasites in migrating blackcaps at a stopover site in N Iberia varied within and between migration periods (autumn/spring). Complementary, we also investigated to what extent infected birds had lower fuel loads.

Material and Methods

Sampling area

This study was carried out at the Loza lagoon (42°50'N 01°43'W, 415 m.a.s.l.), a 50-ha surface with meadows (75-80%), reed beds (10%) and hedgerows and poplar groves (10-15%), placed 5 km W from Pamplona city, 40 km S of W Pyrenees, in N Spain. Blackcaps were captured with mist nets placed across a hedgerow-line composed by Atlantic shrubs (mainly from family Rosaceae and *Sambucus* spp.) and some elms. This hedgerow is a good stopover site for the blackcap (Arizaga et al. 2008, Arizaga & Barba 2009), which finds large amounts of fleshy fruits in autumn, and flowers and insects in spring. Sampling period was conducted for the autumn migration period 2003 (16 September – 15 November) and the spring migration period 2007 (16 March – 30 April). In 2003 we used 60 linear m of mist nets, placed in 4 sets crossing the hedgerow, 3 sampling days a week. In 2007, 126 linear m of mist nets were used, placed in 5 sets crossing the hedgerow, once a week.

Once captured, each blackcap was ringed and its age and sex determined (Svensson 1998). Two age categories were considered: first-year birds (with less than a year; EURING code 3 or 5), and

adults (with more than a year; EURING code 4 or 6). We recorded tarsus length (± 0.1 mm), body mass (TANITA digital balance; ± 0.1 g), fat scores (scaled from 0 to 8, following Kaiser 1993) and moult state (checking if birds were moulting or not).

We took a blood sample of less than 0.05 ml, from jugular vein in randomly selected birds per sampling day. A drop of the blood was extended over individually marked microscope smears, allowed to air dry, and fixed for 1-2 min in ethanol 100% in the laboratory the same day of capture. Until staining, smears were stored in smear boxes.

Haemoparasites quantification

Smears were stained with Giemsa (1/10 v/v) for 40 min. Extra-cellular (extra-erythrocytic) haemoparasites (*Trypanosoma* spp., microfilaria, as well as large intra-erythrocytic cells of *Leucocytozoon* spp.) were searched by scanning 100 fields in half (longitudinally) smear at 400X magnification. The small intra-erythrocytic haemoparasites *Plasmodium* spp. and *Haemoproteus* spp. were detected by scanning the complementary half of the smear at 1000X, at least in 2000 erythrocytes (Godfrey et al. 1987). Thereafter, the intensity of infection was standardised to the number of infected cells per 2000 erythrocytes. All smears were examined by the same author (XE).

Statistics

Within each period (autumn and spring), data were pulled into 15-days time intervals (fortnights), which were hence used as a time unit for the analyses. When possible, at least 20 birds (10 first-year birds, 10 adults) were included into each fortnight. We did not consider moulting birds, since they could have a different body condition (Jenni & Winkler 1994).

First, we studied whether the prevalence varied (1) between periods (autumn/spring), age or sex classes and (2) among fortnights within each period. With this goal, we used (1) a log-linear analysis to see whether the prevalence differed between periods, age and sex classes, and (2) a contingency test to see whether the prevalence varied with time (fortnights) within each period. To analyse whether the intensity of infection varied between periods, age and sex classes, we used a modified two-way ANOVA for non-parametric

data (based on using the ranks of study variable, rather than the real values, Scheirer et al. 1976) on the intensity of infection with period, age and sex as factors. Such an analysis was carried out because the intensity of infection did not fit a normal distribution (Kolmogorov–Smirnov test: $p=0.003$; $n=45$). To test for variations among fortnights we used a Kruskal–Wallis one-way test within each period.

Second, to test for the effect of parasitaemia on fuel load, we performed an ANOVA, or the corresponding non-parametric test (see above for details), on fuel load with the following variables as factors: prevalence (infected and non-infected), period (autumn and spring), age and sex. We estimated fuel load by means of (1) body mass, once controlled for body size (here assessed with tarsus length, Senar & Pascual 1997; see for further details Arizaga & Barba 2009), and (2) fat scores. Body mass fitted the normal distribution (K-S test, $p>0.05$), but not fat scores (K-S test: $p<0.001$). Thus, we used (1) an ANOVA on body mass with tarsus length as a covariate, and (2) a modified ANOVA for non-parametric data on fat scores, as shown above.

Finally, we tested if fuel load was correlated with the intensity of infection. With this goal, we performed Pearson's (body mass) or Spearman's (fat) correlations.

SPSS v.15.0 for Windows was used for statistical analysis; means are given $\pm$ SE.

Results

A total of 132 smears were examined, relative to 78 blackcaps during the autumn migration period, and 54 during spring. Extra-cellular haemoparasites (*Trypanosoma* spp., microfilaria) and large intra-erythrocytic (*Leucocytozoon* spp.) were not detected in any of the smears, but the intra-cellular *Haemoproteus* spp. and *Plasmodium* spp. (Table 1).

Overall, 45 (34.1%) out of the 132 birds were found to be infected, and the log-linear analysis revealed a significant interaction of sex and prevalence (Table 2), with a higher proportion of males being infected (45.5% versus 22.7%). Within each period, prevalence tended to decrease slightly throughout the autumn and more markedly during the spring, although such a tend-

	First-year Birds				Adults			
	Male		Female		Male		Female	
	Y	N	Y	N	Y	N	Y	N
Autumn								
16 Sep – 30 Sep	2	3	4	1	5	1	2	2
01 Oct – 15 Oct	3	3	5	2	2	2	3	0
16 Oct – 31 Oct	4	5	2	1	0	0	7	1
01 Nov – 15 Nov	5	1	4	3	3	2	3	0
Spring								
16 Mar – 31 Mar	2	1	4	0	0	4	1	0
01 Apr – 15 Apr	5	3	1	1	1	2	6	1
16 Apr – 30 Apr	3	2	6	2	1	1	3	1

Tabla 1. Número de currucas capirotadas (*Sylvia atricapilla*) infectadas (Y) y no infectadas (N) por hemosporidios (*Haemoproteus spp.* y *Plasmodium spp.*), en relación a la edad, sexo y periodo de paso en un área de descanso en el N de España.

Table 1. Number of migrating blackcaps (*Sylvia atricapilla*) that were found to be infected (Y) and non-infected (N) by haemosporidians (*Haemoproteus spp.* and *Plasmodium spp.*), in relation to age, sex and period of passage at a stopover locality from N Iberia.

Parameters	Estimation	SE	Z-values	p-values
Constant	0.916	0.632	1.449	0.147
PR	1.435	0.704	2.039	0.041
PE	0.336	0.828	0.406	0.684
AG	0.336	0.828	0.406	0.684
SX	1.099	0.730	1.504	0.132
PR×PE	0.053	0.919	0.058	0.954
PR×AG	-0.246	0.932	-0.264	0.792
PR×SX	-2.534	1.014	-2.498	0.012
PE×AG	0.426	1.051	0.405	0.685
PE×SX	-0.647	1.000	-0.647	0.518
AG×SX	-0.480	0.986	-0.486	0.627
PR×PE×AG	-0.517	1.190	-0.434	0.664
PR×PE×SX	1.692	1.287	1.315	0.188
PR×AG×SX	1.824	1.285	1.420	0.156
PE×AG×SX	0.538	1.286	0.419	0.675
PR×PE×AG×SX	-1.560	1.620	-0.963	0.336

Tabla 2. Parámetros de estimación del análisis log-lineal empleado para estudiar si la prevalencia varió entre periodos, edad y sexo. PR= prevalencia; PE= periodo, AG = edad, SX = sexo. En negrita se indican los valores significativos.

Table 2. Parameter estimation from a log-linear analysis used to analyze whether the prevalence varied between periods, age and sex classes. PR = prevalence; PE = period, AG = age, SX = sex. Significant P values, in bold.

ency was not significant (autumn: $\chi^2_3=0.289$; $p>0.05$; spring: $\chi^2_2=0.329$; $p>0.05$; Fig. 1).

The intensity of infection among infected birds was 2.8 ± 0.5 gametocytes/2000 erythrocytes (range: 0.9 to 14.4 gametocytes/2000 erythrocytes). Most infected birds had low rates of parasitaemia, while few birds had high ones, with this pattern being similar for age and sex classes (Fig. 2). The intensity of infection did not vary between periods and age classes, but between the sexes (period: $\chi^2_1=0.308$, $p>0.05$; age: $\chi^2_1=0.006$, $p>0.05$; sex: $\chi^2_1=6.783$, $p<0.01$; interactions: $p>0.05$). Thus, males were found to be more highly infected than females (mean values: 0.98 ± 0.22 versus 0.94 ± 0.34 ; median values, and 25 and 75% percentiles: 0.00, 0.00-0.98 versus 0.00, 0.00-0.00). However, such a difference was not significant when only

infected birds were considered (period: $\chi^2_1=0.052$, $p>0.05$; age: $\chi^2_1=1.105$, $p>0.05$; sex: $\chi^2_1=2.354$, $p>0.05$; interactions: $p>0.05$). Within each period, the intensity of infection was constant among fortnights (K-W test; autumn: $\chi^2_3=0.289$; $p>0.05$; spring: $\chi^2_2=0.125$; $p>0.05$).

Effect of parasitaemia on fuel load

The parasitaemia did not have any effect on body size-controlled body mass (tarsus length: $F_{1,131}=17.326$, $p<0.001$; prevalence: $F_{1,131}=0.235$, $p>0.05$; period: $F_{1,131}=1.744$, $p>0.05$; age: $F_{1,131}=0.551$, $p>0.05$; sex: $F_{1,131}=0.005$, $p>0.05$; interactions: $p>0.05$), nor on fat scores (prevalence: $\chi^2_1=0.215$, $p>0.05$; period: $\chi^2_1=0.674$, $p>0.05$; age: $\chi^2_1=0.195$, $p>0.05$; sex: $\chi^2_1=0.570$, $p>0.05$;

interactions: $p > 0.05$). Furthermore, fuel load was not correlated with the intensity of infection in birds that were found to be infected (body mass: $r = 0.015$, $p > 0.05$; fat: $r = 0.025$, $p > 0.05$).

Discussion

Prevalence and intensity of infection

Only *Haemoproteus* spp. and *Plasmodium* spp. were detected in a population of migratory blackcaps during the autumn and spring migrations. Extra-cellular species relative to genera *Trypanosoma* spp. and microfilaria, or to large intra-erythrocytic *Leucocytozoon* spp. were not detected, suggesting that in case of being present these haemoparasites would be very scarce at Loza. This result is in contrast with other areas from N Europe, where (1) the community of parasites was reported to change during migration period (reviewed by Valkiūnas 2005), and (2) *Leucocytozoon* spp. was the most abundant haemosporidian with a prevalence of ca. 30% (Valkiūnas 1993).

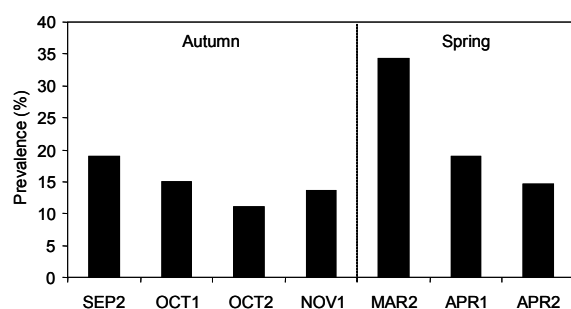


Figura 1. Prevalencia de hemoparásitos en cada estación (quinceñas) durante los periodos migratorios de otoño y primavera.

Figure 1. Prevalence of haemosporidians within each season (fortnights) during the autumn and spring migration periods.

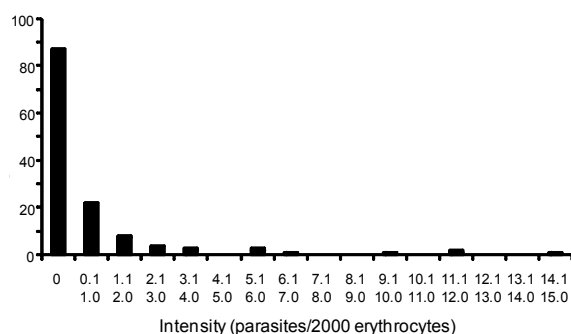


Figura 2. Distribución de frecuencias de hemoparásitos en la población del hospedador.

Fig. 2. Distribution of frequencies of haemoparasites within host population.

It is possible that these differences could be caused by ecological constraints associated with latitude (Valkiūnas 2005 and the references cited there). If we assume that most birds were infected in their breeding areas (Bennett et al. 1974, Garvin & Remsen 1997, Sol et al. 2000, Mendes et al. 2005), we cannot rule out the hypothesis that haemoparasites typical from regions of N Europe, such as *Leucocytozoon* spp., were in a latent state when their avian hosts reached S Europe, hence being absent from blood (Valkiūnas 2005).

We observed a mean prevalence of ca. 35% both during autumn and spring. Although prevalence tended to decrease within each period, the trend was non-significant but we cannot rule out that this lack of differences was due to the relatively low sample size, especially in spring.

Interestingly, males were found to have a higher prevalence rate than females. Such a result suggests that males were more prone to be infected, which could be due to sex-associated immunologic variations (Clayton & Moore 1997).

Prevalence did not vary between age classes, supporting that first-year birds reached similar rates of infection than adults. Similarly, Pérez-Tris & Bensch (2005) did not find differences in prevalence of the *Haemoproteus-Plasmodium* complex between age classes of different European blackcap populations, hence being possible that this pattern is general for the species overall.

The intensity of infection was low overall, with most blackcaps having none or very few haemosporidians and some few individuals having a comparatively high intensity of infection (Anderson and May 1979, Krebs 1999). Infected blackcaps showed a mean intensity of infection of nearly 3 parasites/2000 erythrocytes, a much lower value than that suggested by Valkiūnas (2005) to consider a bird to have a high intensity of infection. Possible hypotheses explaining such a low intensity are (1) highly infected blackcaps were absent from Loza, either because the intensity of infection for the blackcap is commonly low, at least in N Iberia and during the timing of migrations, or because highly infected birds would have lower survival, hence being likely that many of them would have died before arriving to N Iberia. Conversely, it could be possible that highly infected birds were found at Loza, but that they were not detected, either because they are less mobile or because they are more vulnerable to predators

(Valkiūnas 2005). In this case, our results may be biased since we could have captured only those birds in their initial or final phase of their infections (Valkiūnas 2005). Experimental analyses with captive birds could clarify this question.

Effect of parasitaemia on body condition of blackcaps

We did not find any effect of parasitaemia (both in terms of prevalence and intensity of infection) on body condition of migrating blackcaps. Such a result is in accordance with previous data obtained in the same area and demonstrating a null effect of parasitaemia on fuel deposition rate and fuel load of blackcaps during migration period (Arizaga et al. 2009). However, this result must be considered with caution since most birds had low rates of infection and such a low intensity was estimated from counts of *ca.* 2,000 erythrocytes, that could make it difficult to detect any effect (correlation) of parasitaemia on fuel load. In an analysis of more than 3,500 passerines, Bennett et al. (1988) did not find any remarkable effect of haemoparasites on body mass. Such a result can be due to the fact that the majority of wild birds are those with no infections or with chronic ones, whilst birds with heavy infections might be eliminated by natural selection rapidly (Valkiūnas 2005, Møller & Nielsen 2007). Thus, the lack of response in body mass to blood parasites could simply show the host-parasite coevolution, by means of which parasites would not have any relevant impact on fitness of their avian hosts. This process could explain, in addition, the low intensity of infection found in birds captured at Loza (Clayton & Moore 1997).

Acknowledgements

We are grateful to the people who collaborated to collect samples, particularly to D. Alonso, M. Hernández, A. Murua, A. Vilches. J. L. Greño and B. Alonso provided us valuable advices to prepare the smears. J. L. Greño and G. Tomás helped with the identification of some doubtful parasites. The Government of Navarra authorised us to ringing. J. Arizaga was supported by a postgraduate fellowship from the Basque Government. This work was partially founded by the Government of Navarra (to M. Hernández). EB was partially supported by project CGL2007-61395 (Ministry of Edu-

cation and Science, Government of Spain). Two anonymous reviewers provided very valuable comments that improved an earlier version of this work.

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