Effects of migratory status and habitat on the prevalence and intensity of infection by haemoparasites in passerines in eastern Spain

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Abstract

Effects of migratory status and habitat on the prevalence and intensity of infection by haemoparasites in passerines in eastern Spain.— The Iberian peninsula is a suitable place to study the effects of migratory condition on the prevalence of blood parasites in avian communities as resident, local populations cohabit with migratory species and with abundant vector populations. In this study we examined the incidence of avian blood parasites in three localities in the Mediterranean region (east Spain), in relation to the migratory status of the species. We analyzed 333 blood smears from 11 avian species, and obtained an overall prevalence of 9.6%. The prevalence of parasites varied among the different species studied, although intensity of infection did not. Our results are discussed in terms of population dynamics and abundance of Diptera vectors able to transmit blood parasites to other birds.

Key words: Blood parasites, *Trypanosoma* ssp., *Haemoproteus* spp.*, Passeriformes, Diptera vectors.

Resumen

Efectos del estatus migratorio y del tipo de hábitat sobre la prevalencia y la intensidad de la infección por hemoparásitos en paseriformes en el este de España.— La península ibérica es un sitio idóneo para estudiar los efectos de la condición migratoria en la prevalencia de hemoparásitos en comunidades de aves, dado que convergen poblaciones residentes locales con especies migratorias y abundantes poblaciones de vectores. En este trabajo examinamos la incidencia de hemoparásitos presentes en aves de tres localidades de la región mediterránea (este de España), con respecto del estatus migratorio. Examinamos 333 frotis sanguíneos de 11 especies, y encontramos una prevalencia global del 9,6%. A diferencia de la intensidad de la infección, la prevalencia de parásitos mostró variación entre las distintas especies estudiadas. Nuestros resultados se interpretan en relación con la dinámica de poblaciones y la abundancia de dípteros vectores capaces de transmitir los hemoparásitos a otras aves.

Palabras clave: Hemoparásitos, *Trypanosoma* spp., *Haemoproteus* spp., Paseriformes, Dípteros vectores.

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Introduction

Avian hematozoa parasites (Protista) are a heterogeneous group of organisms widely distributed worldwide (Peirce, 1981; Valkiūnas, 2005). Atkinson & Van Riper (1991) noted that haemoparasites have been recorded in almost 70% of the avian species examined, although prevalence estimates may depend on the method used in their detection (Fallon et al., 2005).

Parasites from the genus *Haemoproteus* are among the most common avian haematozoa (two thirds of the described blood parasite morphospecies, Valkiūnas, 2005). Parasites of the genus *Leucocytozoon, Plasmodium* (Bennett et al., 1993) and some *Trypanosoma* species (Kučera, 1982) are also common in avian species. These haemoparasites exert selective pressure on their hosts (Hamilton & Zuck, 1982), negatively affecting the efficiency of metabolism (Chen et al., 2001), survival, breeding success, and physical aptitude (Marzal et al., 2008; Stjernman et al., 2008; Ruiz de Castañeda et al., 2009; Martínez de la Puente et al., 2010), and body growth (Soler et al., 2003).

The incidence of haemoparasites in avian communities varies geographically (Sol et al., 2000). This variation has been linked to habitat characteristics, species composition in the community, vector–host specificity and ecological requirements of the vectors (Deviche et al., 2005). Prevalence and intensity of parasitic infections on birds may also depend on the migratory status of the host species. The probability of being infected would thus be higher in migratory species than in sedentary species, as they are exposed to more than one parasitic fauna during their life cycle (Figuerola & Green, 2000). Migration may also limit the transmission of parasites to new host species, due to the vector–host specificity (Hellgren et al., 2008).

Habitat features affect the incidence of infections in birds (Martínez–Abraín et al., 2004) due to differences in vector abundance and behavior (Bennett et al., 1982). The incidence of parasitemia can thus be expected to be lower in semi–arid regions (Little & Earlé, 1995) than in humid regions with aquatic environments (Moyer et al., 2002).

A latitudinal gradient related to climatic conditions and their effect on vectors could be involved in the prevalence of blood parasites in birds (Bensch & Åkesson, 2003). Several studies carried out in the north and center of Europe, where seasonal climatic changes are severe, found that the prevalence of haemoparasites was relatively high (*i.e.* Kučera, 1981; Valkiūnas et al., 2003; Shurulinkov & Golemansky, 2003), but other studies found a higher prevalence in the south (Marzal el al., 2011). Thus, in southern Europe no clear pattern in the prevalence of blood parasites has been observed (*i.e.* Merino et al., 1997; Valera et al., 2003). Depending on latitude, the Iberian Peninsula shows peculiar climatic characteristics that make it suitable to host high numbers of migratory birds during the winter (Tellería, 1988). Mild peninsular winters thus provide a great variety of resources both for short distance migrants and resident bird populations (Senar & Borras, 2004).
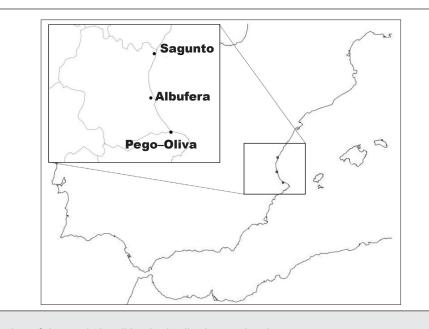
In Spain, a number of significant studies have been carried out to describe and understand the patterns of haemoparasite infections in birds (Merino et al., 1997; Tomás et al., 2007). Studies in the center of the country have shown that the higher the vector abundance, the higher the haemoparasitic prevalence (Merino & Potti, 1995). Preliminary surveys on the Mediterranean coast showed that haemoparasites are almost absent in the Passeriformes species (*Parus major, Periparus ater, Lophophanes cristatus*; E. Barba, non–published data). Similarly, blood parasites were absent in nigh–jars, *Caprimulgus ruficollis* (Forero et al., 1997) and in storks *Ciconia ciconia* (Jovani et al., 2002) in Doñana National Park, a patchy region with wetland and Mediterranean forests. Absence of blood parasites was also noted in Kentish plover and gulls breeding on the Mediterranean coast of Spain (Figuerola et al., 1996; Martínez–Abraín et al., 2002). However, a high prevalence of infection by blood parasites has been found in both migratory and resident species in the south of the Iberian peninsula (Marzal et al., 2008, 2011; López et al., 2011). Most parasitic infections are transmitted by Diptera (Ceratopogonidae, Culicidae and Simuliidae) and the abundance of these vectors depends on the local climate and water conditions in each season (Valkiūnas et al., 2003).

The present study aimed to determine the haemoparasitic infection prevalence and intensity in Passeriformes in three localities in Eastern Spain, to analyze the differences in prevalence and intensity of infection between resident and wintering species, and to relate these measures with the presence of vectors that are potential transmitters of the parasitic infections.

Material and methods

Birds included in this study were trapped between September and December 2008 in three localities close to the Mediterranean coast in eastern Spain (fig. 1). The first locality was the Marjal Pego–Oliva Natural Park (Pego–Oliva; 38° 52' N, 0° 3' W), on the border between the provinces of Valencia and Alicante. The birds were trapped in a wetland with large reed bed areas with mixed patches of cattails and sedge, next to rice fields. The second study site was an orange grove (*Citrus sinensis*) in Sagunto, province of Valencia (Sagunto; 39° 42' N, 0° 15' W), 4 km from the coast. The third locality was L'Albufera Natural Park (Albufera; 39° 19' N, 0° 21' W), a wetland in the south of the Valencia city, dominated by rice fields and some patches of marshland natural vegetation.

In the three study areas, birds were trapped using mist–nets, operating weekly as part of the constant effort ringing programs. In all three sites, 60 m of mist–nets were set at dawn and were operated for 4 hours, following Belda et al. (2007). Each bird was banded with an individual metal ring. Each species was catalogued as resident (species present throughout the year) or wintering (migratory species that winter but do not breed in the study area).

Fig. 1. Location of the study localities in the Iberian peninsula.

Fig. 1. Situación geográfica de las localidades en la península ibérica.

We extracted a drop of blood from the brachial vein of each trapped bird. The drop was placed on a glass slide and dried air. In the laboratory, samples were fixed with absolute methanol and dyed with Giemsa for 45 minutes, following the protocol of Merino et al. (1997). We randomly chose one half of the slide and quantified at 400x the presence of extracellular parasites (*Trypanosoma* spp.) or intracellular parasites (*Leucocytozoon* spp.) along the longitudinal axis. The number of haemotozoa observed in 100 optic fields was recorded. Infection intensity by intracellular parasites (*Haemoproteus* spp. o *Plasmodium* spp.) was obtained as the number of parasites per 2,000 erythrocytes, following Merino & Potti (1995). All the slides were revised by J. R. Parasite identification was based in the morphological characteristics after Valkiūnas (2005).

Complementarily to the bird sampling, water samples were collected in the three study areas to determine the composition of the potential vector community (related to haemoparasite transmission) in different water bodies (such as irrigation ponds, natural springs, and channels). We sampled 52 water bodies, 39 in natural habitats and 13 in artificial ponds. The sampling was performed using a hand net with a square frame of 25 cm per side and a net with pore diameter of 250 µm Each sub–sample was concentrated on a 30 x 40 cm plastic plate. Sampling was concluded when no new taxa were found in the sub–sample. The whole sample (as the set of sub–samples) was stored in a plastic 1 l bottle in 70º alcohol. In the laboratory, samples were washed with water in a 250 µm pore diameter sieve to remove the silt. Species were identified following Tachet et al. (2000) and Rueda & López (2003) using a Motic Digital Microscope DM 143 stereoscopic microscope and a Bresser TrinoLab 40–1,600x microscope.

The prevalence and infection intensity were analyzed at two levels: migratory status and habitat (locality). We analyzed the differences between groups using mixed generalized linear models (GLMMs) fitted by Laplace approximation. Two analyses were made for both *Trypanosoma* spp. and *Haemoproteus* spp. using locality as a fixed factor in the first analysis and migratory status in the second, and individual and species as random factor in both analyses. The individual–level represents a per–observation error term, which captures over–dispersion (Elston et al., 2001; Atkins et al., 2013). We were unable to analyse the two effects together because of zero inflation in the results; and one for *Trypanosoma* sp. only with data obtained in Chiffchaffs (*Phylloscopus collybita*) as it was the only species that was trapped in the three localities (using locality as fixed factor) (Brew & Maddy, 1995). We used binomial models with a logit link function and Poisson models with a logarithmic link function. All tests were performed using the *lme4* package v.0.999375–42 (Bates et al., 2012) for R version 2.14.1 (R Development Core Team, 2009).

Results

A total of 333 birds were trapped, belonging to 11 species and five families. In all three localities, both migratory and sedentary birds were trapped and

Table 1. Infection status for the individuals of the 11 species included in this study: ITry. Infected by *Trypanosoma* spp.; IHae. Infected by *Haemoproteus* spp. Migratory status and locality of capture are given for each species.

Tabla 1. Situación de los individuos de las 11 especies incluidas en el presente estudio respecto de la infección: ITry. Infectado por Trypanosoma *spp.; IHae. Infectado por* Haemoproteus *spp. Para cada especie se indican el estatus migratorio y la localidad de captura.*

| | Number of birds | | | Migratory | |
| | Sampled | Infected by | | status | Locality |
		ITry	IHae		
Sylviidae					
Acrocephalus melanopogon	30	3(10)	0(0)	Resident	Pego–Oliva
Cettia cetti	14	0(0)	0(0)	Resident	Pego–Oliva
Phylloscopus collybita	91	5(5.5)	0(0)	Wintering	Pego–Oliva, Sagunto, Albufera
Sylvia atricapilla	30	1(3.3)	21(70)	Wintering	Sagunto
Sylvia melanocephala	21	1(4.8)	0(0)	Resident	Sagunto
Emberizidae					
Emberiza schoeniclus	45	0(0)	0(0)	Wintering	Albufera
Passeridae					
Passer domesticus	11	0(0)	0(0)	Resident	Albufera
Passer montanus	13	0(0)	0(0)	Resident	Albufera
Turdidae					
Turdus merula	20	0(0)	0(0)	Resident	Sagunto
Erithacus rubecula	30	0(0)	2(6.7)	Wintering	Sagunto
Fringillidae					
Fringilla coelebs	28	0(0)	0(0)	Wintering	Albufera
Total	333	10(3)	23(6.9)		

sampled (table 1). Only 32 birds were infected, with a global prevalence of 9.6% (table 1). The most common parasite was *Haemoproteus* spp., which was identified in 23 birds (6.9%). *Trypanosoma* spp. was detected in 10 birds (3.0%). Only one individual of Blackcap *Sylvia atricapilla* showed both parasites (0.3%). No individual showed infection by *Plasmodium* spp.

The prevalence of infected birds differed between species (χ^2 = 216.5, p < 0.001, df = 10, table 1). Haemoparasites were not detected in six species (Cettia's Warbler *Cettia cetti*, Reed Bunting *Emberiza schoeniclus*, Chaffinch *Fringilla coelebs*, House Sparrow *Passer domesticus*, Tree Sparrow *Passer montanus* and Blackbird *Turdus merula;* table 1). Prevalence did not correlate with the number of samples collected for each species (Spearman's rho; *Trypanosoma* spp.: r = 0.549, p = 0.080; *Haemoproteus* spp.: r = –0.299, p = 0.371). The species that showed the highest abundance of parasites and the highest proportion of infected individuals (n = 22) was the Blackcap,

a species that winters in this area, mainly in shrubs and croplands rather than wetlands.

Trypanosoma spp. infections were detected in Sardinian Warbler *Sylvia melanocephala* (n = 1), Chiffchaff (n = 5), Blackcap (n = 1) and Moustached Warbler *Acrocephalus melanopogon* (n = 3), although in an overall analysis no significant differences were found in the infection prevalence between species (χ^2 = 12.20; p = 0.27; df = 10). Taking Pego–Oliva as reference locality level to calculate the estimators of locality effects, we did not find any statistical differences between localities (Wald χ^2 < 0.001; p > 0.999; df = 2), or between migratory status (Wald χ^2 < 0.001; p = 0.988; df = 1) (see the lower coefficient values of each level compared with the higher SE values showed in table 2). In a partial analysis with data collected on Chiffchaffs (the only species found in the three sampling localities), we did not find differences in the prevalence of *Trypanosoma* spp. between localities (Wald χ^2 < 0.001; p > 0.999; df = 2).

Table 2. Results of the *GLMMs* used to analyze the effects of locality and migratory status on the prevalence of *Trypanosoma* spp. and *Haemoproteus* spp.

Tabla 2. Resultados de los modelos lineales generalizados mixtos utilizados para analizar los efectos de la localidad y el estatus migratorio en la prevalencia de Trypanosoma *spp. y* Haemoproteus *spp.*

		Effect	Estimate	SE	Z	p
Trypanosoma spp.	Locality	Intercept	−14.28	28.75	−0.497	0.619
		Sagunto	−0.14	37.05	0.004	0.997
		Albufera	−17.53	$6.3 \cdot 10^6$	< 0.001	> 0.999
		Pego–Oliva	0.00	–	–	–
	Migratory status	Intercept	−15.14	24.97	−0.606	0.544
		Resident	0.60	39.30	0.015	0.988
		Wintering	0.00	–	–	–
Haemoproteus spp.	Locality	Intercept	−21.80	$6.7 \cdot 10^3$	−0.003	0.997
		Sagunto	17.79	$6.7 \cdot 10^3$	0.003	0.998
		Albufera	−0.000001	$8.7 \cdot 10^3$	< 0.001	> 0.999
		Pego–Oliva	0.00	–	–	–
	Migratory status	Intercept	−3.99	1.60	−2.497	0.012
		Resident	−17.40	$4.2 \cdot 10^3$	−0.004	0.997
		Wintering	0.00	–	–	–

Haemoproteus spp. infections were found only in Blackcaps (*n* = 21) and European Robin *Erithacus rubecula* (*n* = 2), and the prevalence differed between all the species (χ^2 = 205.99; *p* < 0.001; df = 10). *A posteriori* test showed that the differences were due to Blackcaps (χ^2 = 18.30; *p* = 0.05; df = 10). Again taking Pego–Oliva as reference locality level to calculate the estimators of locality effects, we did not find statistical differences between localities (Wald χ^2 < 0.001; *p* > 0.999; df = 2) or between migratory status (Wald χ^2 < 0.001; *p* = 0.997; df = 1) (table 2).

Taking Pego–Oliva as reference locality level to calculate the estimators of locality effects, we did not find statistical differences in the intensity of *Trypanosoma* parasitism between localities (Wald χ^2 < 0.001; *p* > 0.999; df = 2), or between migratory status (Wald χ^2 = 0.006; *p* = 0.936; df = 1) (see coefficient values of each level compared with the higher SE values in table 3). Neither did we find differences in the infection intensity between localities when we considered only the data collected on Chiffchaffs (Wald χ^2 = 0.169; *p* = 0.919; df = 2). In the case of *Haemoproteus* infection, intensity results were similar, showing no differences between localities (Wald χ^2 < 0.001; *p* > 0.999; df = 2), or migratory status (Wald χ^2 < 0.001; *p* > 0.999; df = 1) (table 3).

Table 4 shows the composition of the community of Diptera species potentially acting as a vector for haemoparasite transmission in the three sampling areas. The Pego–Oliva area had the highest richness (13 species and eight genera). In Sagunto, only two species (from two different genera) were detected. Unfortunately, our sampling strategy did not allow comparison of species abundance between species or localities.

Discussion

The intensity and prevalence of infection caused by *Trypanosoma* spp. did not differ between species, migratory status, or locality, as shown previously in studies carried out in the center of Spain (Merino et al., 1997) and north of Europe (Hauptmanova et al., 2006). These results may be attributed to several factors: i) low frequency of individuals infected with this parasite (*i.e.* only five individuals of 91 Chiffchaffs sampled showed infection by *Trypanosoma* spp.); ii) problems with the methodology used for the detection could increase the number of false negatives; Apanius (1991) showed that *Trypanosomas* are not commonly found in peripheral blood, but are abundant in the bone marrow of the infected bird; and iii) as sampling was done in autumn, birds may have had low intensity of infection as they successfully passed the peak period of parasitic infection and thus present residual infection rates (Pérez–Tris & Bensch, 2005; Arizaga et al., 2009).

Haemoproteus spp. was the most prevalent infection, with values similar to those reported in other

Table 3. Results of the GLMMs used to analyze the effects of locality and migratory status on the infection intensity of *Trypanosoma* spp. and *Haemoproteus* spp.: PML. Parasite mean load.

Tabla 3. Resultados de los modelos lineales generalizados mixtos utilizados para analizar los efectos de la localidad y el estatus migratorio en la intensidad de la infección causada por Trypanosoma *spp. y* Haemoproteus *spp.: PML. Carga parasitaria media.*

		Effect	Estimate	SE	Z	p	PML
Trypanosoma spp.	Locality	Intercept	−8.18	3.22	−2.54	0.011	–
		Sagunto	−0.006	4.16	−0.001	0.999	0.04
		Albufera	−18.03	$4.5 \cdot 10^4$	< 0.001	> 0.999	0.00
		Pego–Oliva	0.00	–	–	–	0.05
	Migratory status	Intercept	−9.06	2.93	−3.09	0.002	–
		Resident	0.37	4.62	0.080	0.936	0.04
		Wintering	0.00	–	–	–	0.02
Haemoproteus spp.	Locality	Intercept	−22.76	$1.0 \cdot 10^4$	−0.002	0.998	–
		Sagunto	15.89	$1.0 \cdot 10^4$	0.002	0.999	0.90
		Albufera	−0.0003	$1.3 \cdot 10^4$	< 0.001	> 0.999	0.00
		Pego–Oliva	0.00	–	–	–	0.00
	Migratory status	Intercept	−8.36	3.91	−2.14	0.032	–
		Resident	−17.81	$4.6 \cdot 10^4$	< 0.001	> 0.999	0.00
		Wintering	0.00	–	–	–	0.52

studies, with prevalence around 40% during the autumn (Merino et al., 2000; Pérez–Tris & Bensch, 2005; Arizaga et al., 2009). *Haemoproteus* infection is transmitted to birds by *Culicoides* (Diptera: Ceratopogonidae) (Garvin et al., 2006). The life cycle of this parasite develops rapidly, with asexual reproduction stages in the host (Merino et al., 2004), increasing the probability of infection transmission. Our data suggest that prevalence differed between species, although intensity of infection did not differ between species. Differences could also be attributed to the presence of a species with high infection values (Blackcap), although the importance of other variables such as age, sex, immune state, and season could not be tested due to the low sample size.

Migratory status had a significant effect on the prevalence of *Haemoproteus*. Several studies show that *Haemoproteus* infections in migratory birds are common due to the wide distribution range of the parasite (Waldenström et al., 2002; Pérez–Tris & Bensch 2005). Waldenström et al. (2002) also found evidence of blood parasites as a cost of migration in birds, which may have a considerable impact on the evolution of migration.

The highest prevalence of haemoparasites was recorded in Sagunto, although it was the locality with the lowest richness of vectors. This can be explained by the fact that the vector community is not rich but shows high abundance for some species. It is of note that some authors found that the incidence of haemoparasites is correlated with local abundance of vectors (Merilä et al., 1995; Sol et al., 2000). Therefore, if dipteral vectors have a wide distribution and small habitat restrictions, their local distribution and abundance could increase the presence of haemoparasites in different bird populations. According to this hypothesis, we would also expect a high parasitemia in the resident species. However, our results do not show this parasitemia, so we think that in this case, abundance of vectors does not explain the prevalence of haemoparasites.

We think that these results are due to an effect of the host community and the migratory status of the hosts. Our results show that during the winter, Sagunto hosts several species with high blood parasite prevalence, particularly Blackcaps, a wintering species that was only trapped and sampled in Sagunto. In addition, some studies show that the prevalence of haemoparasites is related to macrohabitat characteristics. For example, Tella et al. (1999) noted that species of birds of prey nesting in forests showed a high prevalence of blood parasites. We did not find a high level of parasitemia in the other two localities, possibly due to the different habitat characteristics, as both were wetlands.

Our results highlight the importance of considering migratory status as a possible factor influencing the prevalence of haemoparasites in bird communities.

Table 4. Composition of the community of Diptera vectors in the three study areas: S. Sagunto; P–O. Pego–Oliva; A. Albufera.

Tabla 4. Composición de la comunidad de dípteros vectores en las tres zonas del estudio: S. Sagunto; P–O. Pego–Oliva; A. Albufera.

	S	P–O	A
Anopheles spp.		+	
Culex pipiens	+	+	+
Culex modestus		+	+
Culex theileri			+
Culicoides spp.		+	
Culiseta subochrea		+	
Culiseta longiareolata	+	+	+
Dasyhelea spp.		+	
Forcipomyia spp.		+	
Ochlerotatus caspius		+	
Ochlerotatus detritus		+	
Simulium reptans		+	
Simulium ruficorne		+	
Simulium velutinum		+	
Total species	2	13	4

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