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Avian Haemosporidian blood parasite infections at a migration hotspot in Eilat, Israel

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Ilan Paperna passed away before the finalisation of the manuscript.

ABSTRACT

Haemosporidian blood parasites are frequent amongst passerines. Though they often do not cause detectable consequences to host health, however, their presence or absence and also their prevalence across host populations may potentially carry meaningful information about the health, stress, body condition and viability of bird individuals or populations. The study of migratory birds captured in Eilat, Israel, allowed us to evaluate the prevalence of blood parasite infections in a wide range of both migrant and resident species in spring (N = 1,950) and autumn (N = 538) of 2004 and 2005. According to blood film microscopy, *Haemoproteus* spp. and *Leucocytozoon* spp. were more prevalent in the spring than in the autumn (0.289, 0.082 vs. 0.132, 0.033, respectively), whilst Plasmodium spp. exhibited a slight opposite trend (0.034, 0.056). All other parasites (such as trypanosomes, microfilaria and haemococcidians) were rare. During the spring seasons, prevalences were significantly higher in migrant than in resident species, whilst this difference was only marginally significant in the autumn. Given that Eilat is a migration hotspot for several Palearctic passerine species, the present descriptive study may hopefully serve to set the baseline values for future long-term epidemiological monitoring.

KEYWORDS

Migratory birds, Haemosporidia, prevalence, *Haemoproteus* spp., *Leucocytozoon* spp., *Plasmodium* spp.

INTRODUCTION

Bird migration is a period of exceptional energy demand, and this metabolic demand is particularly acute for long-distance migrants that cross ecological barriers such as high mountains or extensive areas of water or desert (Alerstam 1994; Berthold 2001). Amongst many other traits of birds, long-distance migration likely affects levels of parasitism because of a negative trade-off between the high energetic costs of migration and those of immunocompetence (Møller & Erritzøe 1998; Norris & Evans 2000).

As the energy stored in fat depositions are critically needed during long-distance migration, migrating birds may downregulate their immune system (Råberg et al. 1998). Therefore, previous authors interpreted increased levels of parasitism as a likely cost of migration (Valkiūnas 1993a; Clarabuch & Gonzalez-Solis 1998).

Further, migratory birds annually move between breeding and wintering habitats, and also temporarily use a wide range of further habitat patches as migratory stopover sites. Thus they necessarily encounter a much greater variety of blood parasites, at least *Haemoproteus* and *Plasmodium* parasites during their annual cycle than resident species (Møller & Erritzøe 1998). Valkiūnas (1993b) hypothesised that in the Palaearctic-African bird migration system, African residents (hosting a particularly diverse pathogen fauna, see Lutz et al. 2015) act as reservoirs of tropical avian blood parasites. Thus overwintering in Africa may increase the infection risk for Palaearctic migrants. Indeed, several African resident and overwintering bird species share genetically identical strains of the genera *Haemoproteus* and *Plasmodium*, indicating that infections readily transmit across host species at African wintering sites (Waldenström et al. 2002).

Transmission most probably occurs through blood-sucking Dipteran vectors; *Plasmodium* spp. are transmitted only by Culicidae, *Haemoproteus (Parahaemoproteus)* spp. by Ceratopogonidae, *Haemoproteus (Haemoproteus)* spp. by Hippoboscidae and *Leucocytozoon* spp. by Simuliidae (Valkiūnas 2005).

Though Haemosporidian infections reduce host fitness to a certain degree (Merino et al. 2000), they tend to exhibit relatively low pathogenicity (as indicated by the high prevalence of chronic infections in apparently healthy hosts) in a veterinary sense. However, their presence or absence and also their prevalence across host populations (the proportion of infected individuals) may potentially carry meaningful information about the host health, stress and body condition. Therefore, long-term monitoring of their epidemiology may contribute to monitoring potential changes in population viability. The aim of this descriptive study is to describe Haemosporidian infection levels of migrant and resident birds captured at Eilat, Israel, that is, a major migration hotspot between Africa and the Palearctic (Frumkin et al. 1995; Yosef & Chernetsov 2004; 2005). Hopefully, these data may serve to set baseline values for future long-term epidemiological monitoring.

1. METHODS

Birds were captured using Heligoland-Rybachy traps at the Bird Sanctuary of the International Birding and Research Center in Eilat (29°33'N, 34 °57'E) in the spring (February to late May) and autumn (October to mid-December) of 2004 and 2005. All birds were ringed with the standard aluminium ring; sex and age, morphometric data and weights were recorded. Blood samples were collected from the brachial veins of birds using heparinised haematocrit capillaries (see Paperna et al. 2007, 2008; Paperna & Yosef 2010). Two samples were taken from each bird, then each sample was smeared onto a clean glass slide. Smears were fixed and air-dried then flooded with absolute methanol and stained for 1 h to 1 h and 15 min with 12% Giemsa (Merck product), in pH 7.4 buffer phosphate (Valkiūnas 2005). All birds were released after sampling. Trapping and sampling took place only during the morning hours at the Center, thus the normal daily fluctuations of Haemosporidians in the blood system (Hawking 1975) could not considerably bias our data.

The entire stained smear was screened at ×1000 magnification to include at least 30,000 erythrocytes. Parasites were identified to the genus level based on the morphology following Valkiūnas (2005) and Valkiūnas et al., (2005).

The data analysed in this study were collected during the normal trapping work conducted at the ringing station, and no special effort was made to trap any particular species. Hence, the data is considered as a random sample representing both migrant and breeding Passerines at Eilat (Paperna et al. 2007). Categorisation as migrants versus residents was based on Shirihai (1996).

Prevalence was compared by Fisher's exact test, their confidence interval were calculated by following the Sterne method (Reiczigel 2003). Two-sample t-tests were two sided, carried out using VassarStats (2016).

2. RESULTS

A total of 2,488 birds were sampled representing 128 species. Blood sampling was conducted during the spring (N=1950) and autumn (N=538) migration seasons of 2004 and 2005. Overall, prevalence was higher in the spring than in the autumn for *Haemoproteus* spp. (0.289, 0.132, respectively, Fisher's exact test, P = 0.000) and *Leucocytozoon* spp. (0.082, 0.033, respectively, Fisher's exact test, P = 0.000), whilst slightly lower in the spring than in the autumn for *Plasmodium* spp. (0.034, 0.056 respectively, Fisher's exact test, P = 0.023). All other blood parasites, such as trypanosomes, microfilaria and haemococcidians, occurred rarely and are not reported here.

The total sample contained 15 species that were represented by ≥10 individuals in the spring and also by ≥10 individuals in the autumn (arbitrary limits) (Table 1). In these particular species, *Haemoproteus* prevalence was higher in the spring in 9, and higher in the autumn in 3 cases, *Leucocytozoon* prevalence was higher in the spring in 6, and in 2 cases in the autumn, and *Plasmodium* prevalence was higher in the spring in 8, and 2 in the autumn. (In all other cases, the prevalence of both the spring and autumn equalled zero and, therefore, were excluded from these comparisons.) The majority of these seasonal differences were not statistically significant (Fisher's exact tests, details not shown). However, taken together all the three major blood parasite taxa, their prevalence was higher in 23 cases in the spring but only in 7 cases in the autumn, a trend that significantly differs from equality (Fisher's exact test, P = 0.005).

Prevalence of the major blood parasite taxa were also compared between migrant and resident species, separately for the spring and autumn seasons. There were 45 migrant and 7 resident species represented by ≥10 individuals in the spring samples. Two-sample t-tests assuming unequal sample variances (VassarStats 2016) indicate that the prevalence of *Haemoproteus*, *Leucocytozoon* and *Plasmodium* was higher in migrant than in resident species (t = −2.83, df = 15.54, P = 0.012; t = −2.24, df = 45.03, P = 0.030; t = −5.52, df = 49.87, P = 0.000, respectively). There were 12 migrant and 5 resident species represented by 10 or more individuals in the autumn samples. A similar difference between migrant and resident species were present but only marginally significant (two-sample t-tests assuming unequal sample variances, t = −1.85, df = 15, P = 0.084; t = −1.99, df = 11, P = 0.072; t = −1.85, df = 11, P = 0.091, respectively).

3. DISCUSSION

The main purpose of our present study is to describe the prevalence of haemoparasitic protozoan infections in birds at Eilat,

Table 1. Bird sample sizes and prevalence of the main blood parasite taxa in bird species that were represented by ≥10 individuals in the spring and/or in the autumn seasons of 2004-2005 at Eilat, Israel. Values in brackets are 95% confidence intervals calculated by Sterne's exact method (Reiczigel 2003). Resident species are indicated by (R), all other species are migrants.

	N	N	Haemoproteus		Leucocytozoon		Plasmodium	
	spring	autumn	spring	autumn	spring	autumn	spring	autumn
Accipiter brevipes	37		0.59(0.43-0.75)		0.49(0.32-0.65)		0.05(0.01-0.18)	
Acrocephalus schoenobaenus	30		0.53(0.35-0.70)		0.00(0.00-0.11)		0.13(0.05-0.30)	
Acrocephalus scirpaceus	35	10	0.20 (0.10-0.37)	0.00(0.00-0.29)	0.00(0.00-0.10)	0.00(0.00-0.29)	0.09 (0.03-0.23)	0.00(0.00-0.29)
Anthus trivialis	35		0.11(0.04-0.27)		0.29(0.15-0.46)		0.03(0.00-0.15)	
Bucanetes githagineus (R)		12		0.00(0.00-0.24)		0.00(0.00-0.24)		0.00(0.00-0.24)
Buteo buteo	56		0.89(0.78-0.95)		0.84(0.72-0.92)		0.02(0.00-0.10)	
Calandrella brachydactyla	19		0.00(0.00-0.18)		0.00(0.00-0.18)		0.00(0.00-0.18)	
Calidris minuta	43	12	0.00(0.00-0.09)	0.00(0.00-0.24)	0.00(0.00-0.09)	0.00(0.00-0.24)	0.00(0.00-0.09)	0.00(0.00-0.24)
Cercotrichas galactotes	26		0.08(0.01-0.25)		0.00(0.00-0.13)		0.19(0.08-0.38)	
Chloris chloris	17		0.06(0.00-0.29)		0.00(0.00-0.20)		0.00(0.00-0.20)	
Coturnix coturnix	32		0.00(0.00-0.10)		0.00(0.00-0.10)		0.03(0.00-0.17)	
Delichon urbica	28		0.39(0.23-0.59)		0.04(0.00-0.17)		0.00(0.00-0.12)	
Emberiza caesia	12		0.08(0.00-0.37)		0.00(0.00-0.24)		0.00(0.00-0.24)	
Emberiza hortulana	11		0.09(0.00-0.40)		0.00(0.00-0.26)		0.09(0.00-0.40)	
Hippolais olivatorum	33		0.85(0.68-0.94)		0.00(0.00-0.10)		0.03(0.00-0.16)	
Hippolais pallida	51		0.76(0.63-0.87)		0.00(0.00-0.07)		0.02(0.00-0.10)	
Hirundo daurica	11		0.00(0.00-0.26)		0.00(0.00-0.26)		0.00(0.00-0.26)	
Hirundo rustica	39		0.05(0.01-0.18)		0.00(0.00-0.09)		0.05(0.01-0.18)	
Jynx torquilla	19		0.05(0.00-0.26)		0.00(0.00-0.18)		0.00(0.00-0.18)	
Lanius collurio	18	34	0.66(0.41-0.84)	0.35(0.20-0.53)	0.06(0.00-0.27)	0.03 (0.00-0.16)	0.06 (0.00-0.27)	0.03 (0.00-0.16)
Lanius nubicus	29	10	0.24(0.12-0.43)	0.10 (0.01-0.47)	0.00(0.00-0.12)	0.00(0.00-0.29)	0.03 (0.00-0.17)	0.00(0.00-0.29)
Lonchura malabarica (R)		15		0.00(0.00-0.22)		0.00(0.00-0.22)		0.00(0.00-0.22)
Luscinia luscinia	11		0.64(0.33-0.86)		0.00(0.00-0.26)		0.00(0.00-0.26)	
Luscinia megarhynchos	10		0.10(0.00-0.45)		0.00(0.00-0.29)		0.00(0.00-0.29)	
Lusciniasvecica	21	49	0.00(0.00-0.16)	0.02(0.00-0.11)	0.00(0.00-0.16)	0.00 (0.00-0.08)	0.10 (0.02-0.30)	0.31 (0.19.0-45)
Merops apiaster	76	11	0.07(0.03-0.15)	0.09(0.00-0.40)	0.03(0.00-0.09)	0.00(0.00-0.26)	0.01 (0.00-0.07)	0.00(0.00-0.26)

continued Table 1. Bird sample sizes and prevalence of the main blood parasite taxa in bird species that were represented by ≥10 individuals in the spring and/or in the autumn seasons of 2004-2005 at Eilat, Israel. Values in brackets are 95% confidence intervals calculated by Sterne's exact method (Reiczigel 2003). Resident species are indicated by (R), all other species are migrants.

	N	N	Haemoproteus		Leucocytozoon		Plasmodium	
	spring	autumn	spring	autumn	spring	autumn	spring	autumn
Merops persicus	10		0.00 (0.00-0.29)		0.00(0.00-0.29)		0.00(0.00-0.29)	
Motacila alba	64		0.30 (0.19-0.42)		0.00(0.00-0.06)		0.05(0.01-0.13)	
Muscicapa striata	11	12	0.73 (0.40-0.92)	0.42(0.18-0.71)	0.00(0.00-0.26)	0.08 (0.00-0.37)	0.18 (0.03-0.50)	0.17 (0.03-0.46)
Oenanthe hispanica	14		0.00 (0.00-0.24)		0.00(0.00-0.24)		0.07(0.00-0.32)	
Oenanthe isabellina	19		0.05 (0.00-0.26)		0.05(0.00-0.26)		0.00(0.00-0.18)	
Oenanthe oenanthe	13		0.15 (0.03-0.43)		0.08(0.00-0.34)		0.08(0.00-0.34)	
Passer domesticus (R)	33		0.00 (0.00-0.10)		0.00(0.00-0.10)		0.00(0.00-0.10)	
Passer hispaniolensis (R)	58	60	0.14 (0.06-0.25)	0.08(0.03-0.18)	0.02(0.00-0.09)	0.00(0.00-0.06)	0.02 (0.00-0.09)	0.00(0.00-0.06)
Passer moabiticus	16		0.19 (0.05-0.44)		0.00(0.00-0.21)		0.06(0.00-0.31)	
Phoenicurus phoenicurus	15	40	0.13 (0.02-0.40)	0.38(0.23-0.54)	0.00(0.00-0.22)	0.03 (0.00-0.13)	0.07 (0.00-0.30)	0.13 (0.05-0.26)
Phylloscopus bonelli	18		0.00 (0.00-0.19)		0.00(0.00-0.19)		0.00(0.00-0.19)	
Phylloscopus collybita	37	26	0.00 (0.00-0.09)	0.00(0.00-0.13)	0.05(0.01-0.18)	0.00(0.00-0.13)	0.00(0.00-0.09)	0.00(0.00-0.13)
Phylloscopus sibilatrix	10		0.30 (0.09-0.62)		0.00(0.00-0.29)		0.10(0.00-0.45)	
Phylloscopus trochilus	12	24	0.25 (0.07-0.54)	0.08(0.02-0.27)	0.00(0.00-0.24)	0.00(0.00-0.14)	0.00(0.00-0.24)	0.00(0.00-0.14)
Prinia gracilis (R)	15		0.00 (0.00-0.22)		0.00(0.00-0.22)		0.00(0.00-0.22)	
Pycnonothus xanthopygos (R)	38	30	0.00 (0.00-0.09)	0.00(0.00-0.11)	0.00(0.00-0.09)	0.00(0.00-0.11)	0.00(0.00-0.09)	0.00(0.00-0.11)
Riparia riparia	22		0.00 (0.00-0.15)		0.00(0.00-0.15)		0.05(0.00-0.22)	
Streptopelia decaocto (R)	29	18	0.34 (0.18-0.53)	0.17(0.05-0.41)	0.00(0.00-0.12)	0.00(0.00-0.19)	0.00(0.00-0.12)	0.00(0.00-0.19)
Streptopelia senegalensis (R)	28		0.00 (0.00-0.12)		0.00(0.00-0.12)		0.00(0.00-0.12)	
Sylvia atricapilla	225	23	0.56 (0.49-0.62)	0.52(0.32-0.72)	0.24(0.18-0.30)	0.04 (0.00-0.21)	0.01 (0.00-0.03)	0.00(0.00-0.15)
Sylvia borin	68		0.49(0.37-0.60)		0.10(0.05-0.20)		0.01(0.00-0.08)	
Sylvia communis	47		0.21(0.11-0.35)		0.02(0.00-0.11)		0.11(0.04-0.23)	
Sylvia curruca	123	15	0.16(0.10-0.24)	0.07(0.00-0.30)	0.01(0.00-0.04)	0.00(0.00-0.22)	0.01 (0.00-0.04)	0.00(0.00-0.22)
Sylvia hortensis	37		0.49(0.32-0.65)		0.05(0.01-0.18)		0.05(0.01-0.18)	
Sylvia melanocephala	13		0.08(0.00-0.34)		0.00(0.00-0.23)		0.08(0.00-0.34)	
Sylvia nisoria	79		0.67(0.56-0.77)		0.04(0.01-0.11)		0.05(0.02-0.12)	
Sylvia ruppeli	19		0.05(0.00-0.26)		0.00(0.00-0.18)		0.11(0.02-0.32)	
Upupa epops (R)	10		0.00(0.00-0.29)		0.00(0.00-0.29)		0.00(0.00-0.29)	

a migration hotspot of outstanding importance for several Palearctic bird species, in 2004–2005. Hopefully, this can serve to set the baseline values for future long-term epidemiological monitoring, especially in case of the most prevalent haematozoan parasites; *Haemoproteus* spp., *Leucocytozoon* spp. and *Plasmodium* spp. A recent study pointed out that similar migration hotspots such as Gibraltar (Mata et al. 2015) may play a role in shaping the distribution of haemoparasites.

Previous authors have already pointed out that migratory species tend to have more prevalent haematozoan infections than residents (e.g. Greiner et al. 1975, Valkiūnas 1993b). Accordingly, resident bird species tend to have less developed antiparasitic defence organs (spleen and the bursa of Fabricius) than migrants (Møller & Erritzøe 1998). As migratory species seasonally switch between two or more biotic environments, such as the breeding grounds, migration stopover sites and wintering grounds, they are exposed to more diverse epidemiological risks than resident species inhabiting a single biotic community all the year round. Apparently, the majority of *Haemoproteus* and *Plasmodium* infections of migratory birds tend to originate from subtropical and tropical wintering areas probably because of the higher diversity of birds, avian pathogens and insect vectors in these areas (reviewed by Valkiūnas 2005). This hypothesis is supported by both of our present finding, that (1) spring birds were more often infected than autumn birds and also that (2) migrant species were more often infected than resident species. Evidently, our comparisons across bird species are affected not only by ecological differences but potentially also by phylogenetic effects (similarity owing to common descent) that need to be taken into consideration by applying advanced comparative methods (Felsenstein 1985). As the present sample size and study design did not allow us to control for phylogenetic effects, here we do not attempt to statistically separate ecological versus phylogenetic effects beyond the phenomena observed.

Alternatively, a seasonal bias in detection efficacy (presuming that active transmission of parasites mostly starts in the spring, making infections more detectable) might have also contributed to the experienced difference between the prevalence in the spring and autumn.

Previous authors made several comparisons between birds captured at breeding versus wintering sites (see, e.g. Greiner et al. 1975; Valkiūnas 2005). However, our study site at Eilat is a typical stopover site for many Palearctic migrants, which prompts a further question. Samples taken at short-term stopover sites may be particularly biased if birds in good condition over-fly the area without landing, whilst sickly birds in poor body condition must necessarily stop and forage for food and water resources. Presently, we do not know whether or not such a bias has affected our samples; however, we hope that the basal values summarised above will help to undertake additional studies on the subject in the region and to answer this question in the future.

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