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Short Communication

Blood Parasites in Passerine Birds in Slovakian East Carpathians

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Summary. Wild birds in two adjacent locations of the Bukovské Vrchy Hills in the East Carpathians (Slovakia) were investigated for blood parasites in the post-breeding period. In 2001, 595 birds were investigated at Ruské. In 2003, 289 birds were investigated in Kurników Beskid. In Ruské, parasites of the genera *Haemoproteus*, *Leucocytozoon* and *Plasmodium* were found at prevalences of 21 %, 6 % and 0.5 %, respectively. In Kurników Beskid, the prevalences of infections with the parasites of genera *Haemoproteus*, *Leucocytozoon* and *Trypanosoma* were 17 %, 0.7 % and 0.3 %, respectively. Parasite species found in blood smears of the birds included *Haemoproteus attenuatus, H. balmorali, H. belopolskyi, H. fringillae, H. picae, H. zosteropis, Leucocytozoon dubreuili, L. fringillinarum, L. majoris* and occasionally also *Plasmodium* sp. and *Trypanosoma* sp. Except for *H. fringillae*, all found species of hematozoa are priority findings for Carpathians. This is the first report of finding of *H. zosteropis* in *Hippolais icterina*.

Key words: *Haemoproteus, Leucocytozoon,* Passeriformes, *Plasmodium, Trypanosoma*.

INTRODUCTION

Blood parasites (hematozoa) are a heterogeneous group of organisms that typically live in the blood of the host during at least some of the stages of their development. Hematozoa have been found in 68 % of birds examined to date (Krone *et al*. 2001). Although hematozoa are wide-spread geographically, their prevalences in different regions are very different. The parasites found most frequently in peripheral blood smears include protozoa of the genera *Haemoproteus*, *Leucocytozoon* and *Plasmodium* (Peirce 1989). Besides this group, a number of other protozoan blood parasites from the genera *Trypanosoma, Babesia*, *Atoxoplasma* and *Hepatozoon* and larval stages of nematodes (microfilariae) have also been described in birds. Findings of these genera in peripheral blood smears are, however, rare (Kučera 1982).

Blood parasites in passerine birds were investigated in Slovakia in the 1970s (Kučera 1981a, b, c, 1982). These reports, however, do not specify the locations where the investigations were made, individual species of birds or the determination of individual species of blood parasites. In the present study, blood parasites in birds from the Slovakian part of the East Carpathians were investigated with the aim to determine the spec-

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trum of the parasite species, their prevalence and the intensity of infections.

MATERIALS AND METHODS

The birds examined were mist-netted for the purpose of ornithological research in two locations in the Bukovské Vrchy Hills. The area is in the north-east Slovakia, and it borders with Poland and the Ukraine. In 2001, investigations were made at the lower border of the forest in the abandoned village of Ruské in the Ruská Kotlina Valley (49° 07´ N, 22°21´ E). The location lies 500 m above sea level. A total of about 200 m of mist-nets were used. The netting and collection of blood samples took place from the end of June to mid-September. In 2003, Kurników Beskid Mountain at the Slovak-Polish border (1000 m above sea level) about 3 km from Ruské was selected for bird monitoring. The nets were installed along a narrow grassy meadow on a ridge surrounded with natural woody growths with a dominant representation of the beech (*Fagus sylvatica*). A total of about 200 m of mist-nets were used. The netting and collection of blood samples took place in the second half of August and in September. In Ruské, a total of 595 birds were examined (499 juveniles, 96 adults) of 33 species, and 289 birds (265 juveniles, 24 adults) of 26 species were examined in Kurników Beskid.

Blood samples used for smears were collected from *vena ulnaris cutanea*. The blood smears were left to dry, then fixed by dipping in methanol, and dried again. In the laboratory, the smears were stained with a combination of stains May-Grünwald and Giemsa-Romanowski using a method according to Pappenheim (Lucas and Jamros 1961).

The smears stained were examined microscopically for the presence of blood parasites. At least 100 000 erythrocytes were examined

Table 1. Blood parasites in wild passerine birds in summer 2001 in Ruské. (H - *Haemoproteus* spp., L - *Leucocytozoon* spp., P - *Plasmodium* spp.)

Host species	Positive/examined	Blood parasites in total	Prevalence (%) of H	L	P
Acrocephalus palustris	0/2	0.0			
Aegithalos caudatus	0/13	0.0			
Anthus trivialis	0/13	0.0			
Coccothraustes coccothraustes	1/1	100.0	100.0	0.0	0.0
Certhia familiaris	0/3	0.0			
Dendrocopos major	0/2	0.0			
Emberiza citrinella	1/8	12.5	12.5	0.0	0.0
Erithacus rubecula	26/118	22.0	14.4	9.3	0.0
Ficedula albicollis	0/2	0.0			
F. hypoleuca	0/2	0.0			
F. parva	0/2	0.0			
Fringilla coelebs	3/5	60.0	60.0	0.0	0.0
Garrulus glandarius	2/2	100.0	100.0	0.0	0.0
Hippolais icterina	4/7	57.1	57.1	0.0	0.0
Lanius collurio	4/16	25.0	6.3	12.5	6.3
Locustella fluviatilis	0/4	0.0			
Luscinia luscinia	3/4	75.0	75.0	0.0	0.0
Muscicapa striata	1/2	50.0	50.0	0.0	0.0
Parus ater	0/1	0.0			
P. caeruleus	0/21	0.0			
P. major	2/54	3.7	0.0	3.7	0.0
P. montanus	0/11	0.0			
Phoenicurus phoenicurus	0/2	0.0			
Phylloscopus collybita	3/84	3.6	1.2	2.4	0.0
P. sibilatrix	2/6	33.3	33.3	0.0	0.0
P. trochilus	1/21	4.8	4.8	0.0	0.0
Prunella modularis	0/5	0.0			
Pyrrhula pyrrhula	2/11	18.2	18.2	0.0	0.0
Saxicola rubetra	0/1	0.0			
Sitta europaea	0/9	0.0			
Sylvia atricapilla	83/125	66.4	60.8	15.2	0.0
S. communis	1/21	4.8	4.8	0.0	0.0
Turdus merula	12/17	70.6	58.8	5.9	11.8
Total	151/595	25.4	21.2	6.2	0.5

Table 2. Blood parasites in wild passerine birds in summer 2003 in Kurników Beskid. (H - *Haemoproteus* spp., L - *Leucocytozoon* spp., T - *Trypanosoma* spp.)

Host species	Positive/examined	Blood parasites in total	Prevalence (%) of H	L	T
Anthus trivialis	0/5	0.0			
Certhia familiaris	0/1	0.0			
Dryocopus martius	0/1	0.0			
Erithacus rubecula	28/143	19.6	19.6	0.0	0.0
Ficedula hypoleuca	0/8	0.0			
F. parva	0/2	0.0			
Fringilla coelebs	2/4	50.0	50.0	0.0	0.0
Garrulus glandarius	1/1	100.0	100.0	0.0	0.0
Glaucidium passerinum	0/1	0.0			
Parus ater	1/1	0.0			
P. caeruleus	0/1	0.0			
P. montanus	0/2	0.0			
Phylloscopus collybita	0/11	0.0			
P. trochilus	1/4	25.0	25.0	0.0	0.0
Prunella modularis	4/21	19.0	14.3	0.0	4.8
Pyrrhula pyrrhula	5/10	50.0	30.0	20.0	0.0
Saxicola rubetra	0/3	0.0			
Sitta europaea	1/5	20.0	20.0	0.0	0.0
Sturnus vulgaris	0/1	0.0			
Sylvia atricapilla	9/21	42.9	42.9	0.0	0.0
S. communis	1/13	7.7	7.7	0.0	0.0
S. curruca	0/1	0.0			
Troglodytes troglodytes	0/7	0.0			
Turdus merula	0/4	0.0			
T. philomellos	0/12	0.0			
T. torquatus	0/6	0.0			
Total	52/289	18.0	17.0	0.7	0.3

Table 3. Intensity of infections of birds with blood parasites in Ruské and Kurników Beskid. (L - low intensity, M - medium intensity, H - high intensity)

	Haemoproteus spp. intensity of infection (% of infected)				*Leucocytozoon* spp. intensity of infection (% of infected)			
	n	L	M	H	n	L	M	H
Ruské	126	40	38	22	37	65	32	3
Kurników Beskid	49	23	23	54	2	100	0	0

in each of the smears (Garvin *et al.* 1993) under magnification of 400×. The quantity of erythrocytes was estimated by calculating their numbers in three squares of the eyepiece grid across the smear (Reauz *et al.* 1999) over an area where the erythrocytes formed a uniform layer without overlapping each other, and by calculating the number of squares. If some parasites were found, their number was calculated in a similar manner per 10,000 erythrocytes. Intensity of infection was expressed in three degrees (Lederer 2000): Infections with species *Haemoproteus* and *Plasmodium* were classified as low if fewer than 0.1 % erythrocytes were affected, as medium if 0.1-0.5% erythrocytes were affected and as high if over 0.5 % erythrocytes were affected. Infections with species *Trypanosoma* and *Leucocytozoon* were classified as low if fewer than 0.02 % erythrocytes were affected, as medium if 0.02-0.1% erythrocytes were affected and as high if the number of erythrocytes affected exceeded 0.1 %.

Table 4. Species of blood parasites found in wild birds in Ruské and Kurników Beskid.

Host	Ruské parasite	Kurników Beskid parasite
Coccothraustes coccothraustes	*Haemoproteus fringillae*	-
Emberiza citrinella	*H. fringillae*	-
Erithacus rubecula	*H. balmorali*	*H. balmorali*
Erithacus rubecula	*H. attenuatus*	*H. attenuatus*
Erithacus rubecula	*Leucocytozoon* sp.	-
Fringilla coelebs	*H. fringillae*	*H. fringillae*
Garrulus glandarius	*H. picae*	*Haemoproteus* sp.
Hippolais icterina	*H. zosteropis*	-
Lanius collurio	*Haemoproteus* sp.	-
Lanius collurio	*L. majoris*	-
Lanius collurio	*Plasmodium* sp.	-
Luscinia luscinia	*H. attenuatus*	-
Muscicapa striata	*Haemoproteus* sp.	-
Parus major	*Leucocytozoon* sp.	-
Phylloscopus collybita	*Haemoproteus* sp.	-
Phylloscopus collybita	*Leucocytozoon* sp.	-
Phylloscopus sibilatrix	*Haemoproteus* sp.	-
Phylloscopus trochilus	*H. fringillae*.	*Haemoproteus* sp.
Prunella modularis	-	*H. fringillae*
Prunella modularis	-	*Trypanosoma* sp.
Pyrrhula pyrrhula	*Haemoproteus* sp.	*H. fringillae*
Pyrrhula pyrrhula	-	*L. fringillinarum*
Sitta europaea	-	*Haemoproteus* sp.
Sylvia atricapilla	*H. belopolskyi*	*H. belopolskyi*
Sylvia atricapilla	*L. dubreuili*	-
Sylvia comunnis	*Haemoproteus* sp.	*Haemoproteus* sp.
Turdus merula	*Haemoproteus* sp.	-
Turdus merula	*L. dubreuili*	-
Turdus merula	*Plasmodium* sp.	-

The genus and species of blood protozoa were determined upon their morphological traits according to Valkiunas (1997). Parasite morphology was studied under 1000× magnification. Measurements were made visually using the eyepiece gauge. In some cases, it was impossible to determine the species because of artificial deformations of cells in the smear.

RESULTS

Blood parasites were found in 25% of the 595 birds examined in Ruské. Parasites of the genera *Haemoproteus*, *Leucocytozoon* and *Plasmodium* were found in 21 %, 6 % and 0.5 % of the birds, respectively (Table 1). In Kurników Beskid, blood parasites were found in 22 % of the 289 birds examined there. Parasites of the genera *Haemoproteus*, *Leucocytozoon* and *Trypanosoma* were found in 17 %, 0.7 % and 0.3 % of the birds, respectively (Table 2).

Intensity of blood parasite infections with individual genera of blood parasites are shown in Table 3. The highest intensity of infection with haemoprotea was found in a juvenile *Erithacus rubecula* netted in Ruské on 4 September 2001 (7.8 % erythrocytes infected) and a juvenile *E. rubecula* netted in Kurników Beskid on 28 August 2003 (5.8 % erythrocytes infected). The highest intensity of leucocytozoon infection was found in an adult *Sylvia atricapilla* netted in Ruské on 14 August 2001, where 244 gametocytes per 100 000 erythrocytes were found. The highest intensity of plasmodia was found in a juvenile *Turdus merula* from Ruské netted on 10 August 2001 (0.9 % erythrocytes infected).

The list of species found includes 6 species of the genus *Haemoproteus* and 3 species of the genus *Leucocytozoon* (Table 4). It was not possible to classify the species of any of the parasite belonging to the genera *Plasmodium* and *Trypanosoma*.

DISCUSSION

Wild birds all over the world except Antarctica are commonly the host to haemoprotozoa of the genera *Haemoproteus*, *Leucocytozoon*, *Plasmodium* (Valkiunas 1997) and *Trypanosoma* (Rintamäki *et al.* 1999). In some seasons their prevalence is very high. In winter, however, when there is no transmission of infection by vectors, blood smears will identify infection in a few birds only, and those infections are of a low intensity (Kučera 1981a, b, c; Valkiunas 1997; Hauptmanová *et al.* 2002). Our summer investigations showed that the most frequently found parasites in both of the locations were those of the genus *Haemoproteus*.

Infection prevalences were different in different bird species. Differences in prevalences between bird species have also been described in other locations (Kučera 1981 a, b, c; Shurulinkov and Golemansky 2002). Such variability may be caused by the specificity of individual species of parasites for specific species, or groups of species, of hosts (Deviche *et al.* 2001), differences in environmental demands of vectors and different exposure of the bird hosts to vectors. The exposure may depend on the time of daily activities of individual species, their type of trophic behaviour, selection of the place for nesting, rest, etc.

We assume that most of the infected birds contracted the infection at the site where they were netted because most of the birds examined were juveniles, who stay around for some time after leaving the nest. However, there is no method available that would allow the determination of the ratio between local birds and those just passing through. Autumn migration of most of bird species examined in the study reaches its peak in the second half of September (Ferianc 1973).

For the Carpathians, all the blood protozoa determined are a priority findings, except for *H. fringillae*, reported from Bieszczady by Dymowska and Żukowski (1968). *H. zosteropis* was found for the first time in *Hippolais icterina* (Sylviidae). Only birds from the family Zosteropidae have been recorded as host for this species to date (Valkiunas 1997). According to Valkiunas (1997), *H. zosteropis* has a wide range of distribution includig Afrotropical, Indomalaysian and Palaearctic realm. It is likely that this species can invade other groups of passerine hosts, e.g. species from the family Sylviidae.

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