Acta Protozool. (2003) 42: 205 - 214

ACTA
PROTOZOOLOGICA

Plasmodium and *Leucocytozoon* (Sporozoa: Haemosporida) of Wild Birds in Bulgaria

Peter SHURULINKOV and Vassil GOLEMANSKY

Institute of Zoology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Summary. Three species of parasites of the genus *Plasmodium* (*P. relictum, P. vaughani, P. polare*) and 6 species of the genus *Leucocytozoon* (*L. fringillinarum, L. majoris, L. dubreuili, L. eurystomi, L. danilewskyi, L. bennetti*) were found in the blood of 1332 wild birds of 95 species (mostly passerines), collected in the period 1997-2001. Data on the morphology, size, hosts, prevalence and infection intensity of the observed parasites are given. The total prevalence of the birds infected with *Plasmodium* was 6.2%. *Plasmodium* was observed in blood smears of 82 birds (26 species, all passerines). The highest prevalence of *Plasmodium* was found in the family Fringillidae: 18.5% (n=65). A high rate was also observed in Passeridae: 18.3% (n=71), Turdidae: 11.2% (n=98) and Paridae: 10.3% (n=68). The lowest prevalence was diagnosed in Hirundinidae: 2.5% (n=81). *Plasmodium* was found from March until October with no significant differences in the monthly values of the total prevalence. Resident birds were more often infected (13.2%, n=287) than locally nesting migratory birds (3.8%, n=213). Spring migrants and fall migrants were infected at almost the same rate of 4.2% (n=241) and 4.7% (n=529) respectively. Most infections were of low intensity (less than 1 parasite per 100 microscope fields at magnification 2000x). *Leucocytozoon* was found in 17 wild birds from 9 species (n=1332). The total prevalence of *Leucocytozoon* was 1.3%.

Key words: haemosporidians, *Leucocytozoon*, *Plasmodium,* prevalence, wild birds.

INTRODUCTION:

Haematozoa of the genera *Plasmodium* and *Leucocytozoon* are common among wild birds in the Northern Hemisphere. Their fauna and morphology are relatively well known in Western and Northern Europe and North America (Pierce and Mead 1977,1978; Kučera 1981a; Bennet *et al.* 1982; Bishop and Bennet 1992; Valkiunas 1997; Krone *et al.* 2001). It is known that vectors of *Plasmodium* are some species of mosquitoes (Culicidae) and the vectors of *Leucocytozoon* are dipterans of family Simuliidae (Valkiunas 1997). Until now little has been known about the distribution and the ecology of *Plasmodium* and *Leucocytozoon*, especially in South-Eastern Europe and the neighbouring areas of the Middle East (Valkiunas 1997, Valkiunas *et al.* 1999). From the Balkans data on these parasites were published for Macedonia (Wülker 1919), Greece (Wenyon 1926) and Bulgaria (Valkiunas *et al.* 1999). In the cited publications the blood parasites found were determined by genus and only in a few occasions by species level. The number of the investigated birds was not high. Only

Address for correspondence: Vassil Golemansky and Peter Shurulinkov, Institute of Zoology, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria; Fax: (3592) 988-28-97; E-mail: golemansky@zoology.bas.bg

3 species of these 2 genera were found in the blood of the wild birds from the Balkans, namely: *Plasmodium matutinum* (Valkiunas *et al.* 1999), *Plasmodim praecox (=Plasmodium relictum)* (Wülker 1919), and *Leucocytozoon fringillinarum* (Valkiunas *et al.* 1999). *Plasmodium* was found in the blood of 5 bird species and *Leucocytozoon* in 10 bird species from the Balkan Peninsula (Wülker 1919, Wenyon 1926, Valkiunas *et al.* 1999).

The aim of this article is to present data on the haematozoan fauna of wild birds in Bulgaria, especially on the parasites of genera *Plasmodium* and *Leucocytozoon* and their prevalence in birds of different species, age, sex and migratory status.

MATERIALS AND METHODS

Blood smears of 1332 wild birds of 95 species (35 families and 12 orders) were studied. The birds were caught during the whole year from 1997 through 2001, mainly in 5 localities in Bulgaria: the Kalimok Biological Station, close to the village of Nova Cherna, Silistra District (933 birds caught); the Chelopechene Fishponds (109); Vrana Campsites (76) and Dragoman Lake (48), Sofia District; Durankulak Lake, Dobrich District (79).

Eighty-seven birds were caught in other regions of Bulgaria (Rupite, Blagoevgrad District; Sofia; Nissovo, Russe District and Atanassovsko Lake, Burgas District). The birds were caught in vertical mist nets, and blood was taken by cutting the longest claw of each specimen. Sex and age determination of the birds was made using the field guide by Svenson (1992). All the birds were ringed and released. The families and the species of birds studied and the prevalence of haematozoa infections in different hosts are shown in Table 9.

From each bird caught, 3 (rarely 2) blood smears were prepared, fixed in methanol for 5 min and stained with Giemsa solution for 50 min. The smears were examined by means of Zeiss microscope under 200, 400 and 2000 magnification. Identification of haemoproteids was performed using the descriptions of Valkiunas (1997). In most of the cases *Plasmodium* parasites were identified to subgenus level and in only 8 cases to species level. The difficulties in species identification of *Plasmodium* were due to the low intensity rates of infection, so the number of the erythrocyte meronts found was low. Bird classification followed Svensson and Grant (1999). To calculate the intensity of invasion, all parasites in 100 microscope fields at magnification 2000x were counted (approximately 4000 erythrocytes). All measurements in the text and the tables are given in micrometers.

To verify the degree of reliability of the data, Fisher's Criterion (F-test) was used (Plochinsky 1970). In these comparisons, the degree of probability (p) and the number of the birds investigated (n) were stated in the text. Fisher's Criterion (φ-method) was chosen because it gives better results than the chi-square test in cases when comparing small percentages (less than 20%) (Plochinsky 1970).

According to their migration status, the birds were divided in 3 groups: (i) long-distance migrants, birds species that spend the winter in Africa, South of the Sahara desert or in India; (ii) close migrants, birds species that spend the winter mainly in the Mediterranean zone, however some populations in Europe are partly resident; (iii) non-migratory (resident) birds spend the winter predominantly in their breeding range. Only some northern populations could move during the autumn and winter periods.

RESULTS

A total of 6 species of *Leucocytozoon* and 3 species of *Plasmodium* were found in the blood of the birds studied. *Plasmodium* was found in 82 birds from 26 species (in 27.3% of all species studied). *Leucocytozoon* was found in 17 birds of 9 species (in 9.5% of all species studied).

Leucocytozoon majoris Laveran, 1902 (Table 1)

Morphology: only round gametocytes were seen. More than half of the parasite's perimeter adheres to the host cell nucleus. The nucleus of the parasite is often diffuse, ellipsoid, but sometimes is clear-cut. The nucleolus could be seen in some cases. Small pieces of host cell protoplast were often visible.

Table 1. Measurements (in μm) of *Leucocytozoon majoris* (n=20).

	Range	Mean	Standard deviation
Length of gametocytes	9.0-5.0	12.3	1.7
Width of gametocytes	8.0-13.0	10.2	1.6
Length of erythrocyte nucleus	20.0-38.0	30.2	5.2
Length of gametocyte nucleus	3.0-6.0	4.2	0.9
Width of gametocyte nucleus	1.5-3.0	2.6	0.9

Hosts: *Acrocephalus schoenobaenus, Turdus merula, Oriolus oriolus.*

Intensity of infection: found in 4 birds. Three of the infections were of very low intensity (below 1.0), and in the fourth an intensity of 2.0 parasites per 100 microscope fields was found.

Localities: Nova Cherna and Vrana. At Nova Cherna it was found in the blood of 2 *A. schoenobaenus* (fall migrants, caught in September 2001) and in *O. oriolus* (local breeding bird, May 1999). At Vrana the parasite was observed in the blood of a local young *T. merula* (June 2001).

Notes: the species is widespread in a great number of passerines from all zoogeographic zones, except Neotropical (Valkiunas 1997).

Leucocytozoon fringillinarum Woodcock, 1910 (Table 2)

Morphology: only round gametocytes could be seen. Less than half of the parasite's perimeter adheres to the host cell nucleus. The width of the host cell nucleus is greater in comparison with the other species of passerine's leucocytozoids, so sometimes it is like a small "cap". The parasite's nucleus is round or ellipsoid, with clear nucleolus.

Table 2. Measurements (in μm) of *Leucocytozoon fringillinarum* (n=6).

	Range	Mean	Standard deviation
Length of gametocytes	9.0-10.5	9.8	0.5
Width of gametocytes	8.0-10.0	9.1	0.9
Length of erythrocyte nucleus	12.0-19.0	14.1	2.6
Length of gametocyte nucleus	2.5-5.0	3.9	0.7
Width of gametocyte nucleus	2.0-3.0	2.3	0.4

Hosts: *Phylloscopus trochilus, Sylvia atricapilla, Anthus trivialis, Turdus merula*.

Intensity of infection: usually lower than 1.0 parasite per 100 microscope fields.

Localities: Nova Cherna and Chelopechene. At Nova Cherna it was found in the blood of fall migrants: *Ph. trochilus* (8.3%, n=36) and *A. trivialis* (4.0%, n=24) and only twice in spring in *T. merula* and *A. trivialis.* At Chelopechene the parasite was found once in spring (May 2001) in the blood of *S. atricapilla.*

Notes: species was found in Bulgaria in *Acrocephalus schoenobaenus* by Valkiunas *et al.* (1999). Distributed in all zoogeographical zones (Valkiunas 1997).

Leucocytozoon dubreuili Mathis et Leger, 1911 (Table 3)

Morphology: only round gametocytes were seen. The host cell nucleus is wider in the ends and narrow in the centre. More than half of the parasite's perimeter adheres to the host cell nucleus. The nucleus of the parasite is clear-cut and ellipsoid in shape.

Hosts: *Turdus philomelos, Turdus merula.*

Intensity of infection: found once in the blood of *T. philomelos* with intensity of 3.0 and once in *T. merula* with intensity of 4.0 parasites for 100 microscope fields.

Localities: Nova Cherna (in *T. philomelos*, spring migrant, April 2000) and Rupite - in a local breeding *T. merula* (May 2000).

Table 3. Measurements (in μm) of *Leucocytozoon dubreuili* (n=6).

	Range	Mean	Standard deviation
Length of gametocytes	10.0-13.5	11.5	0.6
Width of gametocytes	8.5-12.0	10.9	0.9
Length of erythrocyte nucleus	21.0-28.0	24.2	2.9
Length of gametocyte nucleus	2.5-4.0	3.3	0.7
Width of gametocyte nucleus	1.5-3.0	2.3	0.3

Notes: species is common in the Holarctic, Indomalayan and Ethiopian zoogeographical zones and very rare in the Neotropic and Australian zones (Valkiunas 1997).

Leucocytozoon danilewskyi Ziemann, 1898 (Table 4)

Morphology: only oval to irregular in shape gametocytes were observed in a single case of infection. Approximately half of the parasite's perimeter adheres to the host cell nucleus. Small volutine granules are present. The parasite's nucleus is elongated in shape with a clear nucleolus situated close to the nuclear membrane. Pieces of the host cell cytoplasm were often observed.

Table 4. Measurements (in μm) of *Leucocytozoon danilewskyi* (n=6).

	Range	Mean	Standard deviation
Length of gametocytes	12-14.5	13.5	0.5
Width of gametocytes	11.5-4.0	12.8	0.9
Length of erythrocyte nucleus	11-15.5	13.7	3.1
Length of gametocyte nucleus	4.0-5.0	4.4	0.6
Width of gametocyte nucleus	3.0-4.5	4.0	0.4

Host: *Asio otus.*

Intensity of infection: found only once with low intensity (below 1.0).

Locality: Nova Cherna. Found in a local breeding bird (April 2001).

Notes: Elongated gametocytes were described for this species (Valkiunas 1997, Krone *et al*. 2001), but we did not find any. Wülker (1919) found a leucocytozoid parasite in the blood of *Athene noctua* from Macedonia. Probably it was *L. danilewskyi,* because it is the only leucocytzoid described until now in owls. The species is distributed in all zoogeographical zones (Valkiunas 1997).

Leucocytozoon eurystomi Kerandel, 1913 (Table 5)

Morphology: only elongated gametocytes were observed in a single case of infection. The host cell is elongated with sharp ends. Its length varies considerably. The host cell nucleus adheres to the parasite. The parasite's nucleus is oval with a clear nucleolus.

Table 5. Measurements (in μm) of *Leucocytozoon eurystomi* (n=6).

	Range	Mean	Standard deviation
Length of gametocytes	24-27	25.7	2.7
Width of gametocytes	6.0-9.5	8.3	1.1
Length of erythrocyte nucleus	10.5-19	14.6	2.5
Length of gametocyte nucleus	3.0-4.5	3.5	0.9
Width of gametocyte nucleus	2.0-3.0	2.2	0.6

Host: *Coracias garrulus.*

Intensity of infection: found only once with an intensity of 5.0 parasites per 100 fields.

Locality: Nova Cherna (May 1999).

Notes: the species is found in Central and Southern Palearctic, Indomalayan and Ethiopian zoogeographic zones (Valkiunas 1997).

Leucocytozoon bennetti Valkiunas, 1993

Found in the blood of *Coracias garrulus*, caught in May 1997 at Nova Cherna. The intensity was very low, so we did not give a description. Dr. G. Valkiunas made species identification.

Plasmodium (*Haemamoeba*) *relictum* Grassi et Feletti, 1891 (Table 6)

Morphology: trophozoites obviously displace the host cell nucleus. The number of the merozoites in the fully-grown meronts is around 20. Gametocytes and meronts are round to ellipsoid. They change the shape of the infected erythrocyte and displace its nucleus. They can fill the entire host cell cytoplasm in the last stages of their growth. The gametocyte nucleus located centrally. Pigment granules small, dispersed and vary greatly in number.

Table 6. Measurements (in μm) of *Plasmodium relictum* (n=10).

Gametocytes	Range	Mean	Standard deviation
Length	5.0-7.5	6.7	0.3
Width	4.0-6.5	6.1	0.3

Hosts: *Lanius collurio, Passer montanus, Parus major, Panurus biarmicus.*

Intensity of infection: found in 7 birds. Except for 2 of them (in the blood of *Lanius collurio*), the intensity of infection was extremely low (below 1.0).

Localities: Nova Cherna in the blood of spring migrants and locally breeding birds, and at Durankulak in the blood of local breeding *Panurus biarmicus* (July 2001).

Notes: *Plasmodium relictum* is the most common *Plasmodium* species in birds (Valkiunas 1997).

Plasmodium (*Giovanolaia*) *polare* Manwell, 1934 (Table 7)

Morphology: meronts with 6-10 merozoites are situated most often in the polar zone of the infected erythrocyte. They are round or ellipsoid in shape. Gametocytes are with ameboid or entire margins and centrally located nucleus. Pigment granules are between 5 and 10, usually gathered in one of the ends of the parasite. Cases of double infection in 1 erythrocyte were observed.

Table 7. Measurements (in μm) of *Plasmodium polare* (n=10).

Gametocytes	Range	Mean	Standard deviation
Length	9.0-16.0	11.6	1.2
Width	2.0-3.0	2.4	0.3

Host: *Hirundo rustica.*

Intensity of infection: Found once with an intensity of infection of 21 parasites per 100 fields.

Locality: found at Nova Cherna in April 2001.

Notes: so far *P. polare* has been observed in the blood of some passerines of the swallow family - *Hirundo pyrrhonota* and *H. cucullata. H. rustica* was not included in the list of the hosts of *P. polare.* Found in all zoogeographical zones except the Australian (Valkiunas 1997).

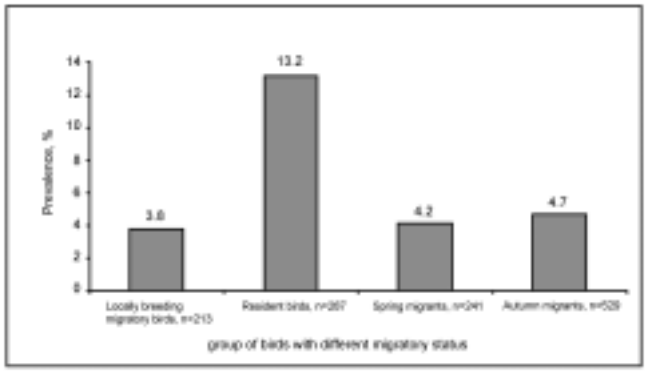

Fig. 1. Prevalence of *Plasmodium* infections of wild birds with different migratory status in Bulgaria.

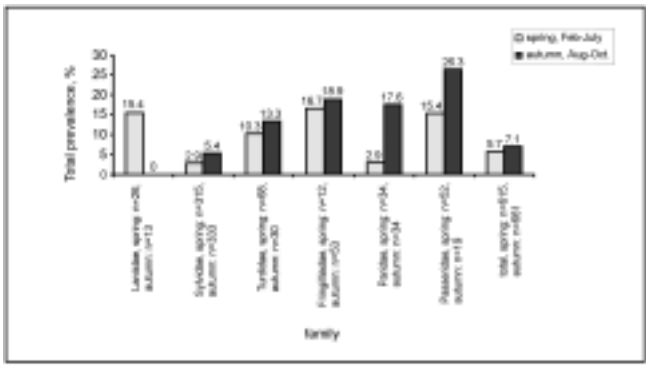

Fig. 3. Seasonal differences in the prevalence of the *Plasmodium* infections among the studied bird families.

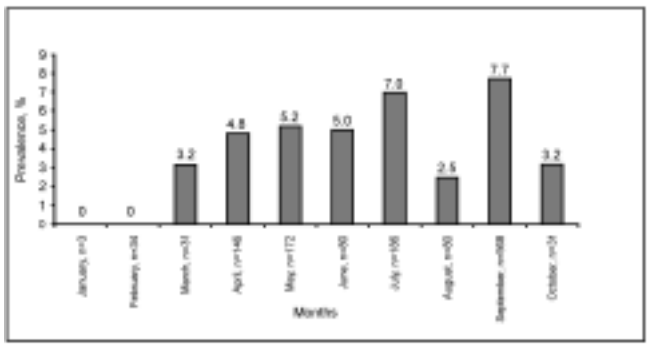

Fig. 2. Total prevalence of *Plasmodium* infections by month.

Plasmodium (*Novyella*) *vaughani* Novy et MacNeal, 1904 (Table 8)

Morphology: meronts are smaller than the erythrocyte nucleus. The number of the merozoites is between 4 and 8. Young gametocytes adhere only to the host cell membrane. Fully-grown gametocytes are elongated with centrally located nuclei. Pigment granules are between 3 and 12 in number, often gathered in a group. The fully-grown gametocytes and meronts do not displace the erythrocyte nucleus.

Table 8. Measurements (in μm) of *Plasmodium vaughani* (n=10).

	Range	Mean	Standard deviation
Length of gametocytes	10.0-12.0	10.8	0.6
Width of gametocytes	1.5-2.5	2.0	0.3
Length of meronts in erythrocyte	2.5-4.0	3.2	0.4
Width of meronts in erythrocyte	1.5-2.5	2.1	0.3

Hosts: *Turdus merula, Sylvia atricapilla, Acrocephalus schoenobaenus.*

Intensity of infection: found in 5 birds with intensity between 1.0 and 5.0 parasites per 100 fields.

Localities: the species was found at Rupite (April 2000) and Vrana (June 2000) in locally breeding birds (*Turdus merula, Sylvia atricapilla*) and at Nova Cherna in fall migrants (*Acrocephalus schoenobaenus*) (September 2000).

Notes: common species, found in all zoogeographical zones (Valkiunas 1997).

Infection prevalence and intensity

The total prevalence in the birds studied was 6.2% for *Plasmodium* and 1.3% for *Leucocytozoon* (n=1332). All *Leucocytozoon* infections were of low prevalence and intensity. *Leucocytozoon* was diagnosed in the blood of 9 bird species: *Asio otus, Coracias garrulus, Anthus trivialis, Turdus merula, Turdus philomelos, Sylvia atricapilla, Acrocephalus schoenobaenus, Phylloscopus trochilus* and *Oriolus oriolus.* Nine cases of infections were found in locally breeding birds (migratory and non-migratory), 6 in fall migrants and 2 in spring migrants.

In Table 9 the prevalence of *Plasmodium* infections in different bird species and families is shown. *Plasmodium* was found only in passerine birds in Bulgaria. It should be emphasized, that the number of passerines studied (1224 individuals), was considerably higher than the number of non-passeriform birds (108 individuals). The highest prevalence was found in Fringillidae (18.5%, n=65), Passeridae (18.3%, n=71) and Turdidae (11.2%, n=98). A low infection rate was found in Hirundinidae: 2.5% (n=81).

Table 9. Number of the examined birds, their migration status and blood parasite prevalence (in %) in different bird families and species. Migration status: Cm - close migrants (spends the winter mainly in the Mediterranean zone), Lm - long-distance migrants, Nm - non-migratory (resident) species.

Bird families/species	Migration status	Examined birds	*Plasmodium*			*Leucocytozoon*		
			Infected birds	Prevalence	Parasite species	Infected birds	Prevalence	Parasite species
Pelecanidae		14						
Pelecanus onocrotalus (Bruch)	Lm	14						
Ardeidae		10						
Ixobrychus minutus (L.)	Lm	10						
Anatidae		1						
Anas platyrhynchos (L.)	Cm	1						
Accipitridae		1						
Accipiter nisus (L.)	Cm	1						
Falconidae		1						
Falco subbuteo (L.)	Lm	1						
Charadriidae		1						
Charadrius dubius (Scop.)	Lm	1						
Scolopacidae		28						
Tringa nebularia (Gunn.)	Lm	1						
Tringa glareola (L.)	Lm	1						
Philomachus pugnax (L.)	Cm	1						
Limicola falcinellus (Pontop.)	Lm	1						
Calidris minuta (Leisler)	Lm	12						
Calidris alpina (L.)	Cm	2						
Calidris ferruginea (Pontop.)	Lm	6						
Arenaria interpres (L.)	Lm	3						
Phalaropus lobatus (L.)	Lm	1						
Laridae		3						
Larus ridibundus (L.)	Cm	2						
Sterna hirundo (L.)	Lm	1						
Columbidae		4						
Streptopelia turtur (L.)	Lm	1						
Columba livia(dom.)(Gm.)	Nm	3						
Cuculidae		5						
Cuculus canorus (L.)	Lm	5						
Strigidae		3				1		
Asio otus (L.)	Nm	1				1		*L. danilewskyi*
Otus scops (L.)	Lm	1						
Athene noctua (Scop.)	Nm	1						
Caprimulgidae		2						
Caprimulgus europaeus (L.)	Lm	2						
Alcedinidae		24						
Alcedo atthis (L.)	Nm	24						
Coraciidae		2				2		
Coracias garrulus (L.)	Lm	2				2		*L. eurystomi*, *L. benneti*
Picidae		12						
Dendrocopus major (L.)	Nm	3						
D. syriacus (Hemp. et Eher.)	Nm	3						
D. medius (L.)	Nm	2						
Picus canus (Gm.)	Nm	2						
P. viridis (L.)	Nm	1						
Jynx torquilla (L.)	Lm	1						
Hirundinidae		81	2	2.5				
Hirundo rustica (L.)	Lm	58	2	3.5	*P. polare*, *P. (Haemamoeba)* sp.			
Riparia riparia (L.)	Lm	18						

Table 9. (contd)

Bird families/species	Migration status	Examined birds	*Plasmodium*			*Leucocytozoon*		
			Infected birds	Prevalence	Parasite species	Infected birds	Prevalence	Parasite species
Delichon urbica (L.)	Lm	1						
Ptyonoprogne rupestis (Scop.)	Cm	4						
Motacillidae		31				2	6.5	
Anthus trivialis (L.)	Lm	25				2	8.0	*L. fringillinarum*
Motacilla flava (L.)	Lm	6						
Prunellidae		5						
Prunella modularis (L.)	Cm	5						
Turdidae		98	11	11.2		5	5.1	
Turdus merula (L.)	Nm	16	2	12.5	*P. vaughani*, *P.* (*Haemamoeba*) sp.	4	25.0	*L. fringillinarum*, *L. dubreuili*, *L. majoris*
T. philomelos (Brehm)	Cm	9	3		*P.* (*Haemamoeba*) sp. *Plasmodium* sp.	1		*L. dubreuili*
T. iliacus (L.)	Cm	1						
Phoenicurus phoenicurus (L.)	Lm	14						
Erithacus rubecula (L.)	Cm	16	1	6.3	*P.* (*Haemamoeba*) sp.			
Saxicola rubetra (L.)	Lm	4						
Luscinia luscinia (L.)	Lm	9	1		*P.* (*Haemamoeba*) sp.			
L. megarhynchos (Brehm)	Lm	29	3	10.3	*P.* (*Haemamoeba*) sp. *P.* (*Novyella*) sp.			
Sylviidae		648	27	4.2				
Sylvia nisoria (Bech.)	Lm	5						
S. borin (Bodd.)	Lm	7						
S. atricapilla (L.)	Lm	53	2	3.8	*P. vaughani* *P.* (*Haemamoeba*) sp.	1	1.9	*L. fringillinarum*
S. communis (Latham)	Lm	15	2	13.3	*P.* (*Haemamoeba*) sp.			
S. curruca (L.)	Lm	20	1	5.0	*Plasmodium* sp.			
Acrocephalus arundinaceus (L.)	Lm	162	8	4.9	*P.* (*Haemamoeba*) sp. *P.* (*Novyella*) sp. *Plasmodium* sp.			
A. palustris (Bech.)	Lm	52						
A. scirpaceus (Herm.)	Lm	65						
A. schoenobaenus (L.)	Lm	173	14	8.1	*P. vaughani*, *P.* (*Haemamoeba*) sp. *P*. (*Giovanolaia*) sp.	2	1.2	*L. majoris*
A. agricola (Jerdon)	Lm	5						
Hippolais icterina (Vieil.)	Lm	5						
Phylloscopus trochilus (L.)	Lm	36				3	8.3	*L. fringillinarum*
P. sibilatrix (Bech.)	Lm	11						
P. collybita (Vieil.)	Cm	5						
Locustella fluviatilis (Wolf)	Lm	9						
L. luscinioides (Savi)	Lm	25						
Muscicapidae		40	2	5.0				
Ficedula parva (Bech.)	Lm	6						
F. semitorquata (Hom.)	Lm	1						
F. albicollis (Temm.)	Lm	3						
F. hypoleuca (Pall.)	Lm	5						
Muscicapa striata (Pall.)	Lm	25	2	8.0	*P.* (*Haemamoeba*) sp.			
Aegithalidae		2						
Aegithalos caudatus (L.)	Nm	2						
Remizidae		7						
Remiz pendulinus (L.)	Cm	7						
Paradoxornithidae		8	1					
Panurus biarmicus (L.)	Cm	8	1		*P. relictum*			

Table 9. (contd)

Bird families/species	Migration status	Examined birds	*Plasmodium*			*Leucocytozoon*		
			Infected birds	Prevalence	Parasite species	Infected birds	Prevalence	Parasite species
Paridae		68	7	10.3				
Parus major (L.)	Nm	47	7	14.9	*P. relictum*, *P.* (*Haemamoeba*) sp. *P.* (*Giovanolaia*) sp.			
P. caeruleus (L.)	Nm	17						
P. palustris (L.)	Nm	4						
Sittidae		5						
Sitta europaea (L.)	Nm	5						
Certhiidae		6						
Certhia brachydactyla (Brehm)	Nm	1						
C. familiaris (L.)	Nm	5						
Troglodytidae		8						
Troglodytes troglodytes (L.)	Nm	8						
Laniidae		42	4	9.5				
Lanius collurio (L.)	Lm	39	3	7.7	*P. relictum*, *P.* (*Haemamoeba*) sp.			
L. minor (Gm.)	Lm	3	1		*Plasmodium* sp.			
Corvidae		3	1					
Pica pica (L.)	Nm	2						
Garrulus glandarius (L.)	Nm	1	1		*P.* (*Giovanolaia*) sp.			
Oriolidae		3	1			1		
Oroilus oriolus (L.)	Lm	3	1		*P.* (*Novyella*) sp.	1		*L. majoris*
Sturnidae		3						
Sturnus vulgaris (L.)	Cm	3						
Passeridae		71	13	18.3				
Passer domesticus (L.)	Nm	29	9	31.0	*P.* (*Haemamoeba*) sp. *Plasmodium* sp.			
P. hispaniolensis (Temm.)	Lm	22	2	9.1	*P.* (*Haemamoeba*) sp.			
P. montanus (L.)	Nm	20	2	10.0	*P. relictum*			
Fringillidae		65	12	18.5				
Coccotrhaustes coccothraustes (L.)	Nm	2						
Fringilla coelebs (L.)	Cm	14	3	21.4	*P.* (*Haemamoeba*) sp.			
Carduelis chloris (L.)	Nm	30	7	23.3	*P.* (*Haemamoeba*) sp.			
C. carduelis (L.)	Cm	19	1	5.3	*P.* (*Haemamoeba*) sp.			
Emberizidae		22	1	4.6				
Emberiza hortulana (L.)	Lm	1						
E. schoeniclus (L.)	Cm	17						
E. citrinella (L.)	Nm	1						
Miliaria calandra (L.)	Cm	3	1		*P.* (*Haemamoeba*) sp.			
Total		1332	82	6.2		17	1.3	

Plasmodium infections were most common in local non-migratory birds (Fig. 1). The total prevalence for these birds was 13.2% (n=287), which is higher than the rate established for the local migratory birds: 3.8% (n=213) (F-test, $p<0.001$). There was no significant difference in the prevalence between fall migrants: 4.7% (n=529) and spring migrants: 4.2% (n=241) (F-test, $p>0.05$). The prevalence in local migratory birds and transitory migrants (both fall and spring) did not differ significantly. The prevalence for the close migrants was 5.8% (n=65) and for the long-distance migrants - 4.1% (n=975). The difference was not significant (F-test, $p>0.05$). The migration status of each bird species is shown in Table 9.

Plasmodium parasites of the subgenus *Haemamoeba* were most commonly found (61.9% of all infections). Other subgenera were rarely found: *Novyella* (9.5%) and *Giovannolaia* (4.8%). About 23.8% of the *Plasmodium* infections were not determined to subgenus level.

The seasonal distribution of the *Plasmodium* infections showed small monthly variations (Fig. 2). Nevertheless a trend of increasing the rates from March to September could be marked. The highest prevalence was found in September (7.7%).

The comparison between the average prevalence for spring and summer (from March until the end of July) and the autumn prevalence (August-October) (Fig. 3) showed that in all studied families, except Laniidae, the autumn rates were higher. Differences were not significant (F-test, $p>0.05$), except for the family Paridae, where a significant difference was found (F-test, $p<0.05$).

Plasmodium infection rates did not differ significantly between males (10.2%, n=124) and females (11.2%, n=130) (F-test, $p>0.05$).

Adults and young birds (<1- year old) in summer and autumn periods (June - October) showed almost the same prevalence: 7.8% (n=293) for the adults and 7.4% (n=402) for the young birds (F-test, $p>0.05$). In this comparison the birds with difficult age determination are excluded.

The infection intensity of *Plasmodium* was usually low or very low (less than 1 parasite in 100 microscope fields at magnification 2000x). Part of the infections of the lowest intensity was probably missed. So the total *Plasmodium* prevalence given, 6.2%, is lower than the real one. Intensive infections with more than 10 parasites in 100 fields were found in 5 birds (4 of them in spring and summer).

Four cases (in 4 bird species) of mixed infections with 2 subgenera of *Plasmodium* were found: in the blood of *Parus major, Haemamoeba* and *Giovannolaia*; in *Fringilla coelebs, Haemamoeba* and *Giovannolaia* or *Novyella*; in *Acrocephalus schoenobaenus, Haemamoeba* and *Giovannolaia* or *Novyella*; and in *Turdus merula - Haemamoeba* and *Novyella*. In the last case *Leucocytozoon dubreuili* was also found. Similar cases have been reported frequently by other authors (Valkiunas 1997).

Mixed invasions of *Plasmodium* and other haemosporidians were also found: 9 cases of *Plasmodium/Haemoproteus* (one or 2 species) type and one case of *Plasmodium/Leucocytozoon* type. One adult *Oriolus oriolus* infected with 4 parasites was caught: *Plasmodium* (*Novyella*) sp., *Haemoproteus orioli, Leucocytozoon majoris* and *Trypanosoma* sp. (29.05.1999, Nova Cherna). A similar case was found in *Fringilla coelebs* infected with *Plasmodium* (*Haemamoeba*) sp., *Haemoproteus fringillae, H. dolniki* and *Trypanosoma* sp. (14.06.2000, Vrana campsite). Three cases of mixed infections of *Plasmodium/ Hepatozoon* type were also found (2 of them in *Parus major* and 1 in *Acrocephalus schoenobaenus*). A total number of 18 (22%) mixed infections of *Plasmodium* (n=82) were diagnosed.

DISCUSSION

The number of *Plasmodium* and *Leucocytozoon* species known in Bulgaria (4 and 6 respectively) is considerably lower than expected until now. For *Plasmodium* this is due to the difficulties in the species identification, especially in the cases with low rates of intensity of infection. At Curonian Spit in the Baltic Sea 11 species of *Plasmodium* and 15 of *Leucocytozoon* were described (Valkiunas 1997).

The total prevalence of *Leucocytozoon* in Bulgaria (1.3%) is lower than in Northern and Central Europe. At Curonian Spit the prevalence was 12.2% (Valkiunas 1985); in Poland, 12.4 - 13.0% (Dymowska and Żukowski 1965, 1968; Ramisz 1965); in Central Europe, 6% (Kučera 1981a). In Southeastern Kazakhstan (a region on a similar geographic latitude as Bulgaria), a total prevalence of 2.2% was found (1.13% only for the passerines) (Jakunin 1972). These data are similar to ours.

In contrast to the *Leucocytozoon* the *Plasmodium* infection rates were on average higher in Bulgaria than in most of the other regions in Eurasia (Manwell 1956, Jakunin 1972, Pierce and Mead 1976, Valkiunas 1985). In Central Europe Kučera (1981b) found a higher total prevalence of *Plasmodium* in bird groups that were not included in our investigation (orders Galliformes and Columbiformes). Higher infection rates than these in Bulgaria were found in Africa: 13.6% in Egypt (Helmy Mohammed 1958) and 13.0% in Morocco (Gaud and Petitot 1945).

A comparison between *Plasmodium/Leucocytozoon* prevalence in birds of different migratory status was made by many authors (Ramisz 1965; Kučera 1981a, b; Valkiunas 1987, 1997). Likewise, in the present study, a high total prevalence of *Plasmodium* infections for the local non-migratory birds was also found in Poland

where up to 81% of *Plasmodium* infected birds were locally breeding non-migratory birds (Ramisz 1965). Kučera (1981b) also found in Central Europe a higher prevalence of *Plasmodium* in the non-migratory birds compared with the long distance migrants. It is our opinion, that the main reason for the lower *Plasmodium* prevalence found for the long-distance migrants in the present study is the high number of non-infected autumn migrants (mainly young birds) in our sample. These birds belong to the populations from Northern parts of Europe and Asia, where there is no local transmission of malarial parasites (Valkiunas 1997).

Ramisz (1965), Jakunin (1972) and Kučera (1981b) mentioned that the levels of parasitemia for *Plasmodium* were higher in spring and summer than in autumn. Not only the high number of the vectors could cause this spring peak in this period but also by spring relapses of parasitemia (Valkiunas 1997, Krone *et al.* 2001). According to Valkiunas (1997) the best weather conditions for the transmission of the malarial parasites in Holarctic could be reached only in the end of the summer period. Probably this could explain the higher *Plasmodium* prevalence found by us for family Paridae, including mostly locally breeding, resident birds. The higher *Plasmodium* prevalence in September, compared with August, found in the present study, could be connected with more favourable (more humid) weather conditions for the vectors (dipterans of family Culicidae) during September. In Bulgaria August is usually very dry month, which is not the case in September.

In some publications (Kučera 1981b, Valkiunas 1987, Hatchwell *et al.* 2000), it was shown that adult birds are more often infected with *Plasmodium* than are young birds (<1-year old). But in other studies just the opposite data were gathered (Bennet and Fallis 1960, Jakunin 1972). Our data showed almost equal rates of parasitemia for adults and young birds. This proportion probably varies in different groups of birds and in different local populations.

Acknowledgements. We would like to thank Dr. G. Valkiunas and Dr. T. Iezhova from the Institute of Ecology of the Lithuanian Academy of Sciences for their kind help in the identification of some of the observed haemosporidians. We also thank Dr. P. Zehtinjiev and Dr. D. Pilarska from the Institute of Zoology of the Bulgarian Academy of Sciences for their field assistance in the preparation of a part of the studied blood smears.

REFERENCES

Bennet G. F., Fallis A. M. (1960) Blood parasitism of birds in Algonqiun Park, Canada, and a discussion of their transmission. *Can. J. Zool.* **38:** 261-273

Bennet G. F., Whiteway M., Woodworth-Lynas C. B. (1982) A host parasite catalogue of avian haematozoa. Occasional papers in biology, No 5, Memorial University of Newfoundland

Bishop M., Bennet G. F. (1992) Host - parasite catalogue of the avian haematozoa: Supplement 1, and Bibliography of the blood-inhabiting haematozoa: Supplement 2. Occasional papers in biology, No 15, Memorial University of Newfoundland

Dymowska Z., Żukowski K. (1965) Pierwotniaki pasożytnicze w materiale sekcyjnym z ptaków puszczy Kampinowskiej. *Wiad. parazytol.* **11:** 477-481 (in Polish)

Dymowska Z., Żukowski K. (1968) Pierwotniaki krwi ptaków odłowionych na terenie Bieszczadów. *Wiad. parazytol.* **14:** 455-459 (in Polish)

Gaud J., Petitot M. (1945) Hematozoaires des oiseaux du Maroc. *Arch. Inst. Pasteur Maroc*. **3:** 149-171

Hatchwell B. J., Wood M. J., Anwar M., Perrins C. M. (2000) The prevalence and ecology of the haematozoan parasites of the European blackbirds, *Turdus merula. Can. J. Zool.* **78:** 684-687

Helmy Mohamed A. H. (1958) Systematic and Experimental Studies on Protozoal Blood Parasites of Egyptian Birds. Cairo University Press, Vol. **1**

Jakunin P. (1972) Blood parasites of wild birds from South-eastern Kazakhstan. *Pap. Inst. Zool. AN Kazakh. SSR* **33:** 69-79 (in Russian)

Krone O., Premier J., Sömmer P., Langemach T., Lessow O. (2001) Haemosporida of birds of prey and owls from Germany. *Acta Protozool.* **40:** 281-289

Kučera J. (1981a) Blood parasites of birds in Central Europe 2. *Leucocytozoon. Folia parasitol.* (*Praha*) **28:** 193-203

Kučera J. (1981b) Blood parasites of birds in Central Europe 3. *Plasmodium and Haemoproteus. Folia parasitol.* (*Praha*) **28:** 303 -312

Manwell R. D. (1956) Blood parasitism in the English sparrow, with certain biological implications. *J. Parasitol.* **43:** 428-433

Pierce M. A., Mead C. (1976) Haematozoa of British birds 1. Blood parasites of birds from Dumfries and Lincolnshire. *Bull. B. O. C.* **96:** 128-132

Pierce M. A., Mead C. (1977) Haematozoa of British birds 2. Blood parasites of birds from Hertfordshire. *J. Nat. Hist.* **11:** 597-600

Pierce M. A., Mead C. (1978) Haematozoa of British birds 4. Blood parasites of birds from Wales. *J. Nat. Hist.* **12:** 361-363

Plochinsky N. (1970) Biometry. Moskow University Press, Moscow (in Russian)

Ramisz A. (1965) Zależność występowania pasożytów krwi ptaków wróblowatych w Polsce od wędrowek oraz sezonowych zmian temperatury. *Wiad. parazytol.* **11:** 467-476 (in Polish)

Svensson L. (1992) Identification Guide to European Passerines. Singraph AB S. O. D. E. R. T. A. L. J. E.

Svensson L., Grant P. (1999) Bird Guide. Harper Collins Publ.

Valkiunas G. (1985) Blood parasites of birds from Belomoro-baltijsky stream of migration. 2. Fauna and distribution of haemoproteids (Sporozoa, Haemosporida) *Parazitologia* **19:** 55-63 (in Russian)

Valkiunas G. (1987) Blood parasites of birds from Belomoro-baltijsky stream of migration. 4. Ecological aspect. *Parazitologia* **21:** 537-544 (in Russian)

Valkiunas G. (1997) Bird Haemosporida. *Acta zool. Lithuanica* 3-5 (monography)

Valkiunas G., Iezhova T., Golemansky V., Pilarska D., Zehtindjiev P. (1999) Blood protozoan parasites (Protozoa: Kinetoplastida and Haemosporida) in wild birds from Bulgaria. *Acta zool. Bulg.* **51:** 127-129

Wenyon C. M. (1926) Protozoology. Bailliere, Tindall and Cox, London, Part **2**

Wülker G. (1919) Über parasitische Protozoen Mazedoniens. *Arch. Schiffs Tropenhyg.* **23:** 425-431

Received on 13th November, 2002; revised version on 7th March, 2003; accepted on 16th April, 2003