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PREFACE

The 31st international conference Ecological Truth & Environmental Research – EcoTER'24 focuses on showing the latest research findings and innovations in the field of ecology, environmental protection and sustainable development. The conference will be held in Sokobanja (Serbia) in hotel Sunce in the period of 18–21 June 2024.

The aim of the conference is to connect the experts in various fields in order to transform attitudes and behaviors in everyday practices, as well as in the industry and economy sector which is essential for achieving the desired changes that our society must undergo.

The 31st international conference Ecological Truth & Environmental Research – EcoTER'24 is organized by the University of Belgrade, Technical Faculty in Bor, and co-organized by the University of Banja Luka, Faculty of Technology; the University of Montenegro, Faculty of Metallurgy and Technology – Podgorica; the University of Zagreb, Faculty of Metallurgy – Sisak; the University of Pristina, Faculty of Technical Sciences – Kosovska Mitrovica and the Society of Young Researchers – Bor.

These Proceedings encompass 119 papers from the authors coming from the universities, research institutes and industries in 15 countries: Brazil, Norway, USA, Spain, Austria, Libya, Italy, Israel, Slovenia, Croatia, Romania, Bulgaria, Montenegro, Bosnia and Herzegovina, North Macedonia, and Serbia. It is a great honor and pleasure to cordially wish a warm welcome to all the participants of the conference.

As a part of this year's conference, the 6th Student Section – EcoTERS'24 will be held. We appreciate the contribution of the students and their mentors who have also participated in the conference and hope that students will continue to explore and to be curious, since education is a never-ending process, and knowledge is continuously growing.

The organization of the EcoTER'24 conference has been financially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia.

The support of the Donors and their willingness and ability to cooperate has been of great importance for the success of the EcoTER'24 conference. The organizing committee would like to extend their appreciation and gratitude to the Platinum donors of the conference – Serbia ZiJin Copper doo Bor and HBIS SERBIA, to the Gold donor of the conference – Elixir Group, as well as to the Silver donor of the conference – Serbian Chamber of Engineers.

We would like to express our sincere appreciation to all the authors who have contributed to the Proceedings. We would also like to express our gratitude to the members of the scientific, organizing and honorary committees, reviewers, speakers, chairpersons and all the conference participants for their support of the EcoTER'24. Sincere thanks go to all the people who have contributed to the successful organization of the EcoTER'24.

Prof. Snežana Šerbula,

President of the scientific and organizing committee

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HAEMOSPORIDIAN PARASITES IN LONG-EARED OWLS WINTERING IN BANAT, SERBIA

Daliborka Stanković[1*], Draženko Z. Rajković[1], Marko Raković[1], Stefan Skorić[1]

[1]University in Belgrade-Institute for Multidisciplinary Research, Kneza Višeslava 1, 11000 Belgrade, SERBIA

[*]*daliborka@imsi.bg.ac.rs*

Abstract

Parasites from the Phylum Apicomplexa (genera Haemoproteus, Plasmodium and Leucocytozoon) are the vector-born blood parasites distributed worldwide. This group of parasites has been the subject of extensive study in passerines, but less so in other groups of birds, such as owls. Four haemosporidian species have been discovered in the Long-eared Owl Asio otus so far. As there are only a few records of haemosporidian infection in wintering Long-eared Owls, in our pilot project we studied the prevalence, parasitemia, and diversity of the blood parasites of owl mentioned above in Banat, Serbia. We found the presence of all three genera, of which Leucocytozoon was the most abundant. The total prevalence was 52.2%, while the average parasitemia in winter was low, only 0.16%. We also observed two cases of co-infection. Interestingly, the presence of parasites did not affect the SMI (scaled mass index) and the health status of the birds.

Keywords: haemosporidians, prevalence, long-eared owls, *Asio otus*.

INTRODUCTION

Haemosporidian parasites from the Phylum Apicomplexa (genera *Haemoproteus*, *Plasmodium* and *Leucocytozoon*) are spread across all zoogeographical regions, excluding the Antarctic [1]. This group of vector-born parasites and their avian hosts is a very dynamic and complex system [2]. While some haemosporidian parasites are capable of infecting multiple host species [3–5], others are highly host-specialised [6–8]. Although parasites and their hosts have a long co-evolution history and adaptation, the clinical signs of infection caused by haemosporidians vary among individuals. In general, they can negatively affect feather growth, cause anemia, weight loss, negative body condition, or may even lead to detrimental effects [9–11].

Most haemosporidian studies have been conducted on passerines, while owls have been widely neglected [12,13] despite their important role in ecosystems [14]. So far, eight species of avian Haemosporidia have been discovered and described in order Strigiformes [1] with more than 298 unique lineages worldwide [15]. In the Long-eared Owl *Asio otus*, four haemosporidian species were detected, including seven *Haemoproteus*, one *Plasmodium* and 12 *Leucocytozoon* lineages [15]. The prevalence of the Long-eared Owl varies between 40% and 75% in Germany [12,16] up to 82% in the USA [13]. While in the most research *Leucocytozoon* tended to be the most common genus, Martín-Maldonado *et al*. [17] in Spain found *Haemoproteus* to be the only parasite infecting the Long-eared Owl with parasitemia

up to 12.8%. In Vojvodina (Serbia), Minichová *et al*. [18] detected all three haemosporidian genera in Long-eared Owl, with *Leucocytozoon* being the most abundant.

The Long-eared owl is a well-known, widespread, medium-sized owl. It breeds throughout northwest Africa, Europe, Asia and North America, except in the far north and south. Four subspecies have been recognised, of which the nominal inhabits mainland Europe [19]. It is a regular breeder in Serbia, mainly in Vojvodina [20], but also in the mountain regions [21]. It breeds mostly in old Corvid nests [20]. The national breeding population is estimated at 19,000–28,000 breeding pairs [22]. The presence of a large number of Long-eared Owls wintering in the Province of Vojvodina (Serbia) [23], allows us to sample a significant number of owls in the roost sites and to easily study haemosporidian parasites. However, no study on Long-eared Owl haemosporidian parasitemia has been conducted so far.

The aim of the present study was to assess the diversity, prevalence and parasitemia of blood parasites in Long-eared Owls wintering in Banat, Serbia. Additionally, we explore the potential impact of these parasites on the SMI (scaled mass index).

MATERIALS AND METHODS

Study sites and sample collection

The study was conducted in December 2023 at four localities (Taraš, Mokrin, Padinska skela and Opovo) in Banat, in Vojvodina region. Birds were captured while leaving the roosting trees using ornithological mist nets (mesh size 70 mm). They were ringed, aged, and measured with standard protocols [24]. To assess the physical condition of the birds we used the scaled mass index (SMI), as it appears to be a reliable predictor [25]. SMI is based on the scaling relationship between mass and wing/tarsus length, which was measured and then used for further calculation the SMI. We also collected a small amount of blood from each bird by puncturing the brachial vein. A small drop of blood was used to prepare a thin blood smear, air dried and later fixed in 96% ethanol for 5 minutes according to Valkiūnas [1]. After bleeding was stopped the owls were safely released.

Slides examination

In the laboratory, blood smears were stained with Giemsa solution as recommended by Valkiūnas [1]. Slides were examined by LEICA DMLS light microscope [26] in order to estimate the prevalence and intensity of infection (parasitaemia). As described, parasitemia was estimated as a percentage by counting the number of parasites per 1,000 or 10,000 examined red blood cells, depending on the level of parasitemia [1,27].

Statistical analysis

To examine within-individual differences, Welch Two Sample t-test was used for fitted for infection status, and sex, using the R package version 4.2.2. [28]. The regression slope was 0.34 and the averaged wing length was 297.7 mm, from which using the equation of the linear regression a scaled mass index (SMI) was calculated [25].

RESULTS AND DISCUSSION

In the present study, we screened 23 Long-eared Owls for haemosporidian parasites during the wintering season in 2023, in Banat, Serbia. Even the highest prevalence in owls was recorded during the spring and summer season [17] some study recorded infection in winter as well [18,29]. Indeed, in our study we found haemosporidian infection in Long-eared owls during the autumn-winter period.

To detect haemosporidian blood parasites, we used only the method based on examination of blood smears [1]. We were able to identify parasites morphologically only to the genus level. Previous studies in the Long-eared owls revealed very high prevalence of the blood parasites varying among 70% and 75% [13,16]. In the USA, the prevalence was even higher, up to 82 % [13], while we found that it was 52.2% (Table 1).

Table 1 *Birds infected with haemosporidan parasites, as indicated by blood smears*

Sampling place	No. of birds sampled per site	No. of infected birds	*H*	*P*	*L*	*MI*[*]
Taraš	1	0	-	-	-	-
Mokrin	1	0	-	-	-	-
Padinska Skela	7	2	1	1	-	-
Opovo	14	10	(2)[*]	1 (1)[*]	7 (1)[*]	2
Total	**23**	**12**	**1**	**2**	**7**	**2**

[*]MI - 2 mixed infections (*Haemoproteus*/*Plasmodium* and *Leucocytozoon*/*Haemoproteus*); H - *Haemoproteus*; P – *Plasmodium*; L - *Leucocytozoon*.

So far, in Long-eared owls all three haemosporidian genera with only 4 species have been discovered [1]. Genus *Leucocytozoon* was isolated in the non-captive owls [13,16,18,30], and described as *L. danilewskyi* [1]. In the present study, we found all three genera, with *Leucocytozoon* being the most abundant (Figure 1), while Martín-Maldonado *et al*. [17] detected just *Haemoproteus* infection. According to Valkiūnas [1] and MalAvi database [15], from Strigiformes were isolated two *Haemoproteus* species: *H. syrnii* and *H. noctuae* and one *Plasmodium*: *P. subpraecox*. In our study, *Plasmodium* and *Haemoproteus* participated equally with 13%.

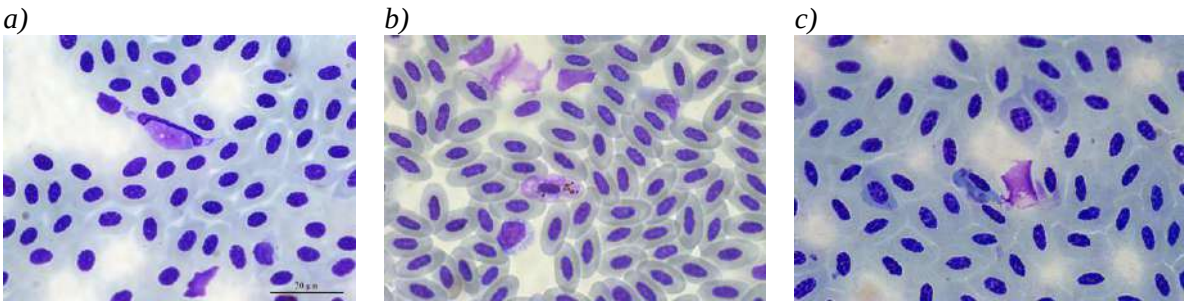

Figure 1 *Genera of Haemosporidian parasites found in the blood smears of the Long-eared Owl a) Leucocytozoon; b) Haemoproteus; c) Plasmodium*

Since Martín-Maldonado *et al*. [17] conducted the study during the breeding season, when vectors are most active, recorded parasitemia in the Long-eared owls was very high, up to 12.8%. Average parasitemia in our study in winter was low, only 0.16%. Co-infections of two or more haemosporidian parasite species occur frequently in all avian species [1] including owls, with the most common combination *Leucoytozoon-Hemoproteus* [13,17]. We also recorded two mixed infections. One bird hosted *Haemoproteus* and *Plasmodium* parasites, while the other was infected with *Leucocytozoon* and *Haemoproteus* species.

Out of all Long-eared owls, 12 were females and 11 were males. There was no significant difference in parasitemia between sex (t-test = -0.97, p-value = 0.35) or age (t-test = 0.12, p-value = 0.9) of the birds, which agrees with Martín-Maldonado *et al*. [17] study. Additionally, as a reliable predictor of the physical condition of birds we used SMI [25]. In this sense, we compared the level of parasitemia with SMI to identify whether the presence of parasites affects the health status of the birds, because a low level of parasitemia depends on bird health, low stress levels and the absence of other pathogens. Therefore, parasitemia can be considered a reliable indicator of birds' health [31]. However, there was no significant difference (t-test = -1.48, p-value = 0.17) between infected and uninfected birds and their physical condition.

As PCR is a more sensitive method for detecting parasite *cytochrome b* gene [32], it will be a crucial tool in the next phase of our research. Moreover, we believe that designing more suitable primers for owl haemosporidian lineages will be necessary to delve deeper into the parasite fauna and gain a better understanding of their phylogenetic place as well as molecular differences, thereby contributing to the broader field of avian health and parasitology.

CONCLUSION

We studied the diversity, prevalence and parasitemia of haemosporidian parasites in wintering population of Long-eared Owls from Banat, Serbia. Additionally, we compared the level of parasitemia with SMI to identify whether the presence of parasites affects the health status of the birds and their effect on SMI. The overall prevalence was 52.2% with the genus *Leucocytozoon* being the most abundant in the sampled birds. *Plasmodium* and *Haemoproteus* participated equally in the study. No significant difference was found between parasitised and non-parasitised birds and their physical condition. In order to gain a better insight into the parasite lineages in the next step of the study, we plan to expand the research to a wider area with more individuals. Additionally, we will use PCR to detect the parasite cytochrome b gene to achieve a better insight of the parasite lineages.

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The samples involved in this study comply with the current legislation of the Ministry of Environmental Protection of the Republic of Serbia.

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