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Prevalence and New Host Records of Avian Blood Parasites in Songbirds from the West of Iran: Insights into Haemosporidian Infections and the Impact of Humidity

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Abstract: Haemosporidian parasites, including *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*, are intracellular parasites found in the blood and tissues of birds worldwide. The prevalence of these parasites varies depending on bird species and habitats. Humidity, influenced by water availability, is one of the factors affecting their prevalence. In this study, we collected 145 blood samples from wild songbirds in the western region of Iran from April to November 2021-2022. Blood smears were prepared using the push-slide method and stained with Giemsa. The stained smears were examined under a light microscope. Among the seven songbird families sampled, blood parasites were detected in 31 individuals from five families. Of these, 23 individuals (15.86%) were from humid areas, while 8 individuals (5.51%) were from dry areas. The highest infection rates were observed in the Paridae (9.65%) and Motacilliidae (4.82%) families, while the lowest infection rates were found in the Corvidae (1.37%) and Acrocephalidae (1.37%) families. We recorded two new hosts for *Leucocytozoon spp*. (*Motacilla flava* and *Acrocephalus melanopogon*) for the first time in the world and four new host records (*Motacilla flava*, *Pica pica*, *Acrocephalus melanopogon*, and *Acrocephalus agricola*) for blood parasites in the west of Iran. Our findings highlight the high prevalence of avian blood parasites in songbirds inhabiting humid areas, suggesting that these environments provide suitable conditions for the growth and reproduction of invertebrate hosts.

Keywords: blood parasites; Iran; *Leucocytozoon spp.*; songbirds

1. Introduction

Blood parasites, specifically haemosporidians, are highly prevalent infections in birds, surpassing the prevalence observed in other vertebrates. The global expansion and substantial prevalence rates of various haemosporidian species highlight the need for their identification and classification. These parasites are primarily transmitted through biting insects such as *Simulium* and *Culex*, with Passeriformes being particularly susceptible compared to other bird orders [1,2]. The life cycle of avian blood parasites is complex, involving both sexual (gametogenesis and fertilization) and asexual (sporogony) reproduction in the invertebrate host, and asexual reproduction (merogony) in the vertebrate host [3–5]. Avian malaria-like diseases are caused by *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* in birds [6]. *Haemoproteus spp*. is considered one of the most significant genera of parasitic organisms and can infect over 50% of bird hosts worldwide [2]. *Leucocytozoon spp*. is also prevalent among wild birds [7]. *Plasmodium spp*., with its more than 200 species in 14 subgenera, includes five subtypes of avian malaria [8]. Among bird species, songbirds have been found to have the highest prevalence of haemosporidian infections. For example, in Bulgaria, songbirds exhibited an infection rate of 58.04% [8], while in Austria, the rate was 43.7% [9]. Similar studies conducted in Iran reported a prevalence of haemosporidian infections of 35.75% in northern regions [10] and 51.1%

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in southern parts of Iran [11]. The prevalence and transmission of blood parasites depend on factors such as vector presence and bird population density [12]. The absence of suitable habitat for vectors of haemosporidian in arid environments has been suggested as a reason for the absence of infections in those areas [13,14]. For example, black flies (family: Simulidae), which are common vectors of *Leucocytozoon spp*., require clean water for reproduction [15,16], making it challenging to establish infections in dry and semi-arid environments. Conversely, mosquitoes and midges, which are vectors for haemosporidian parasites,, require standing water for reproduction, increasing the probability of infection in ponds, lakes [17–19], slow rivers [4], stream margins, fens, and bogs [20]. Another study, by Krama et al. (2015) found that the prevalence of haemosporidian infections decreased with increasing distance from forest lakes [21]. Despite these findings, no studies have yet been conducted on the prevalence of haemosporidian parasites in songbirds in western Iran, nor have there been any comparisons of infection rates between humid and arid areas. Therefore, the aim of this study is to evaluate the prevalence of blood parasites in songbirds in western Iran using light microscopy and investigate whether the prevalence of these parasites in songbirds is related to environmental humidity.

2. Materials and Methods

In this study, a total of 145 blood samples were collected from 145 songbirds representing nine species, seven genera, and seven families. The sampling took place in 13 different localities in the western region of Iran including Sanandaj (35°15′21.97′′ N-47°00′58.86′′E, 1385 m above sea level), Sarvabad (35°18′04.75′′N-46°21′32.85′′E, 1086 m above sea level), and Marivan (35°31′31.21′′N-46°08′55.30′′E, 1287 m above sea level). The sampling period extended from April to November 2021-2022, and the songbirds were captured using mist nets. To collect blood samples, an insulin syringe was used to draw blood from the brachial vein of each bird. The collected blood was then used to prepare smears using a push-slide method. After preparing the smears, they were air-dried, fixed with absolute methanol for five minutes, and dried again. Subsequently, the smears were stained with Giemsa stain at a pH of 7.1 for a duration of 20-25 minutes. The blood spreads were examined under a light microscope, following the method described by Valkiūnas (2005) [1].To determine the humid and dry areas, the method proposed by Krama et al. (2015) was followed. Humid areas were identified based on the presence of rivers and lake margins, while dry areas were selected at least 4 kilometers away from any rivers or lakes. To assess the significant difference in blood parasite infection rates between individuals in humid and dry areas, we conducted a Fisher Exact Test ($p < 0.05$) using SPSS version 22.0 (SPSS for Windows Inc., Chicago, Illinois, 2013) [22].

3. Results

In our study, we found that out of 145 host individuals, 31 (21.37%) were infected with avian blood parasites. Among the positive individuals, 14 birds were found to be infected with two genera (Co-infection) of blood parasites, namely Haemoproteus and Leucocytozoon. The overall prevalence of avian blood parasites was determined to be 30.33%, with Haemoproteus accounting for 17.93%, Leucocytozoon for 11.03%, and Plasmodium for 1.37% of the infections (Figure 1). Interestingly, we observed that 23 individuals (15.86%) were found in humid areas such as Sirwan River and Zarivar Lake, while 8 individuals (5.51%) were detected in dry areas within the city of Marivan and Sanandaj. Importantly, based on the Fisher Exact Test, we found a significant difference (sig= 0.00 < 0.05) in the prevalence of blood parasites between humid and dry areas. The highest infection rate was observed in species belonging to the Paridae (9.65%) and Motacilliidae (4.82%) families, while the lowest infection rate was observed in the Corvidae (1.37%) and Acrocephalidae (1.37%) families (Table 1). Among the 145 captured host songbirds, we recorded two new hosts for Leucocytozoon spp. (Motacilla flava and Acrocephalus melanopogon), which represents the first documentation of their infection worldwide (Figure 1C). Additionally, we identified four new hosts in the western region of Iran, including Motacilla flava, Pica pica, and Acrocephalus melanopogon for Leucocytozoon spp., Pica pica and Motacilla flava for Haemoproteus spp., and Acrocephalus agricola for Plasmodium spp. (Table 1). Furthermore, we detected 14 cases (9.65%) of co-infection with Haemoproteus spp.

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and Leucocytozoon spp., with the Paridae family exhibiting the highest co-infection rate (eight individuals), while the Acrocephalidae family had the lowest co-infection rate (one individual).

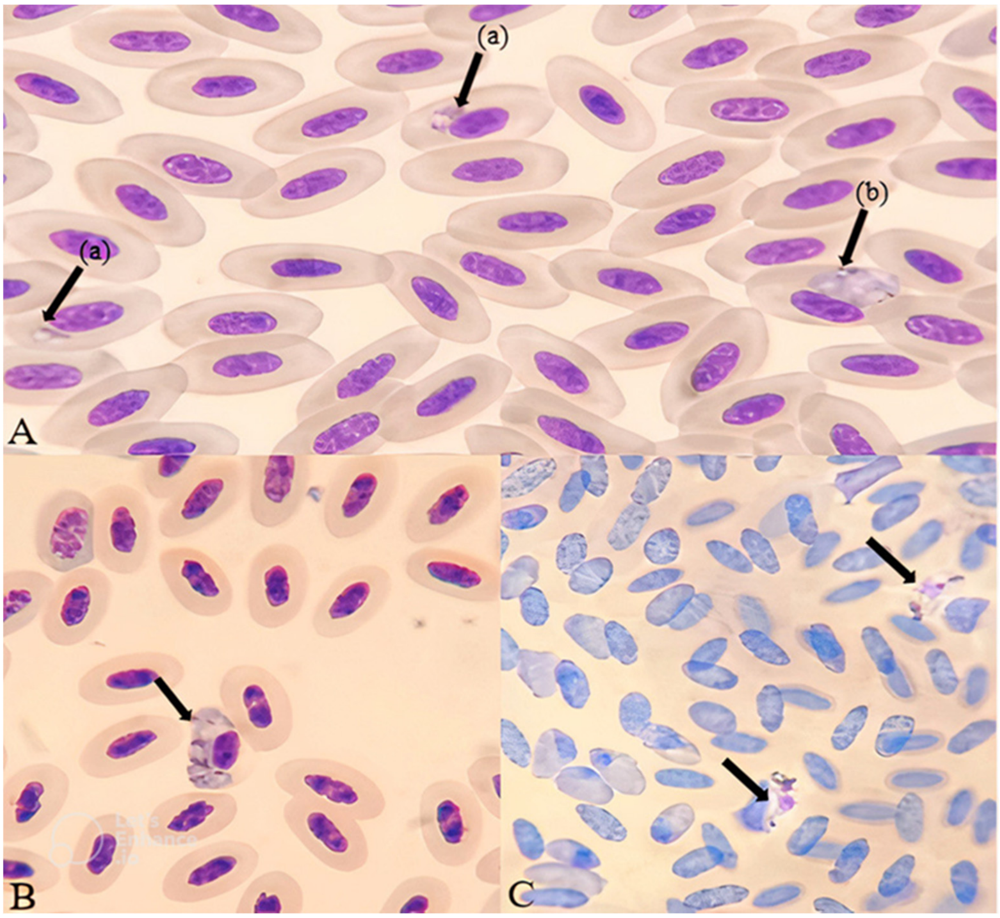

Figure 1. A) Early (left (a)) and late (right (b)) trophozoites of *Plasmodium spp*. in *Acrocephalus agricola*, B): *Haemoproteus spp*. in *Motacilla flava*, and C) *Leucocytozoon spp*. in *Motacilla flava*. (Stained with the Giemsa stain, Magnification 1000×).

Table 1. Prevalence of the avian haemosporidian parasite in songbirds from the west of Iran.

Family and Species for Host	NEx	N Inf	Inf (%)	*H*	*P*	*L*	*Hm*	*Pm*	*Lm*
Corvidae									
Pica pica+	4	2	50%	2	0	2	2	1	2
Pycnonotidae									
Pycnonotus leucotis	1	0	0 %	0	0	0	0	0	0
Passeridae									
Passer domesticus	74	6	8.10 %	3	1	2	16	40	12
Motacilliidae									
*Motacilla flava**+	10	7	70 %	7	0	3	5	6	0
Acrocephalidae									
Acrocephalus agricola+	3	1	33.33 %	0	0	0	5	5	0
*Acrocephalus melanopogon**+	1	1	100 %	1	0	1	1	2	0
Turdidae									
Turdus merula	1	0	0	0	0	0	10	25	22
Paridae									
Parus major	22	6	27 %	5	0	4	18	18	48

Cyanistes caeruleus	29	8	27.58 %	8	0	4	20	22	26
Total	145	31	------	26	2	16			
Prevalence, %		21.37	----	17.39/ 1.37 /11.03					

[1] **NEx** = Number examined, **N Inf** = number infection, **Inf %** = Infection %, ***H*** = *Haemoproteus* spp.; ***P*** = *Plasmoduim* spp.; ***L***= *Leucocytozoon* spp, ***Hm***= number of a recorded lineage of *Haemoproteus* in MalAvi database, ***Pm***= number of a recorded lineage of *Plasmoduim* in MalAvi database, ***Lm***= number of a recorded lineage of *Leucocytozoon* in MalAvi database, + New host records for Iran, * new host record for the world.

4. Discussion

This study showed a range of infection rates among different avian species, with the highest prevalence of *Haemoproteus* parasite infection being 17.93% and the lowest prevalence of *Plasmodium spp*. infection at 1.37%. The overall prevalence of infection varied between 8.10% and 100% across different avian species (Table 1). Notably, *Cyanistes caeruleus* had the highest infection rate (eight individuals), followed by *Motacilla flava* (seven individuals) and *Parus major* (six individuals). Additionally, *Motacilla flava* showed the highest prevalence of *Haemoproteus spp*., while *Parus major* and *Cyanistes caeruleus* showed the highest prevalence of *Leucocytozoon spp*.

Our findings indicated that humid areas had the significantly highest infection rates with haemosporidian parasites (Figure 2). These results align with a study conducted in Baikal Lake, where the prevalence of blood parasites was reported to be as high as 63.9% [23]. Similarly, high prevalence rates were observed in north-western Siberia (76.3%) [24], Bulgaria (58.04%) [8], Lake Superior in the USA (54%) [25], and southern Iran (51.1%) [11]. In contrast, dry areas demonstrated lower prevalence rates of haemosporidian parasites (5.51%) (Figure 2), which is consistent with a study conducted in Almeria City (3.45%) [26].

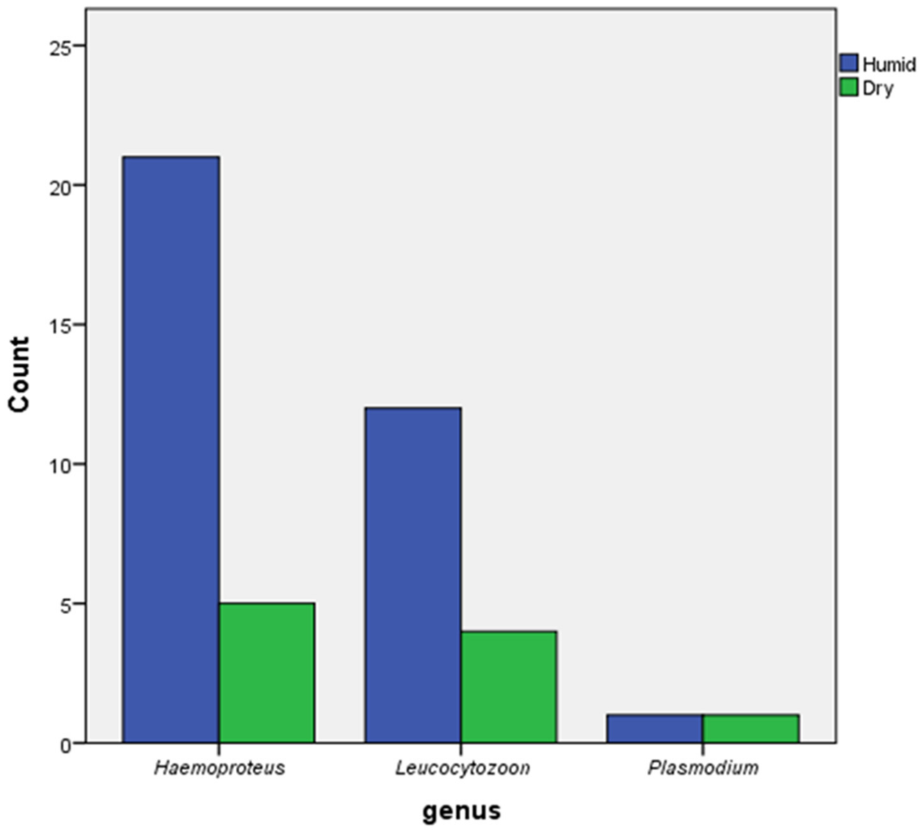

Figure 2. Comparison between individuals infected with haemosporidian parasites in humid (blue) and dry (green) areas in this study.

The overall prevalence of infection in our study aligns with the findings in north Iran [10], which may be due to similar weather conditions between the regions. However, the high prevalence in

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southern Iran [11] can be attributed to the relatively high temperature and humidity in that area. Furthermore, our study found a high prevalence of *Haemoproteus spp*., consistent with other studies [8,11]. However, some studies have reported *Leucocytozoon spp*. as the most prevalent blood parasite in subarctic regions, likely due to the higher abundance of *Leucocytozoon* hosts in those areas [25], or due to higher elevations and colder conditions [27–32]. Temperature is known to be a determining factor for the prevalence of *Plasmodium spp*., typically more prevalent in hot areas [33]. However, our study and the findings in north Iran [10] revealed a low infection rate with the *Plasmodium* parasite (1.37% and 1.26%, respectively), contrasting with the higher rates observed in Bulgaria (24.7%) southern Iran (14.5%) and Austria (13.8%) [8, 9, 11]. In humid areas of our study, the infection rates for *Haemoproteus spp*. and *Leucocytozoon spp*. were higher compared to dry areas, while the infection rate for the *Plasmodium* parasite was similar in both dry and wet areas (Figure 2). This suggests that the prevalence of *Plasmodium spp.* is more influenced by temperature.

In humid areas, we observed 12 cases of co-infection with *Haemoproteus spp*. and *Leucocytozoon spp*., while there were only two cases in dry areas. The highest occurrence of co-infection was found in *Parus major* and *Cyanistes caeruleus*, while *Acrocephalus melanopogon* had the lowest occurrence. However, we did not observe any co-infection between *Haemoproteus spp*. and *Plasmodium spp*., or between *Plasmodium spp*. and *Leucocytozoon spp*. This finding is consistent with previous studies [10, 11].

The overall co-infection rate of 14 individuals in our study aligns with the results of a study conducted by Ghaemitalab et al. (2021), which reported 13 cases of co-infection with *Haemoproteus spp*. and *Leucocytozoon spp*. [11]. However, in the study by Nourani et al. (2018) only one sample was found to be co-infected with *Haemoproteus spp*. and *Leucocytozoon spp*. [10]. The higher occurrence of co-infection in the Paridae family in our study is consistent with a study by van Rooyen et al. (2013), which reported a high incidence of co-infection in great tits [34]. In sum, our results demonstrate a high prevalence of haemosporidian parasites and co-infection in humid areas, indicating that suitable environmental conditions for vectors may play an important role. In future research, it would be valuable to investigate the impact of temperature and other factors on blood parasite infection in these areas.

5. Conclusions

In conclusion, our study reveals a higher prevalence of haemosporidian parasite infections in songbirds residing in humid areas compared to dry areas. This difference can be attributed to the greater abundance and movement of blood parasite vectors in humid regions. Furthermore, our findings suggest that humidity plays a significant role as a determining factor in the prevalence of these parasites.

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Institutional Review Board Statement: This study was carried out according to the protocol approved by a committee on the ethics of animal experiments of the Ferdowsi University Mashhad (Ethical Certificate No. IR.UM.REC.1400.372). Furthermore, all field works were carried out with permits approved by the Department of the Environment. (Permits No. 2/1177/ 1401).

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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