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Genetic variation of Plasmodium and Haemoproteus parasites in birds of Iran

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ABSTRACT

Type: Review Article Background: Plasmodium and Haemoproteus are common parasites Received: 2023/04/17 transferred to birds by Culicidae, Ceratopogonidae, and Hippoboscidae Accepted: 2023/7/19 vectors. These parasites are of particular concern to healthcare researchers and veterinarians due to their impact on avian malaria, association with other animals and humans, and phylogenetic link with human Plasmodium species. *Corresponding Author: Leila Nourani Methods: This bibliographic review article was designed to deliver a Address: Malaria and Vector Research report on the avian malaria infections of *Plasmodium* spp. and Group (MVRG), Biotechnology Research Center (BRC), Pasteur Haemoproteus spp. in birds in Iran. Published reports in Google Scholar, Institute of Iran, Tehran, Iran PubMed, and Science Direct were used for this revision until April 2023. \succ **Results:** The highest number of recorded infections with *Haemoproteus* le.nourani90@gmail.com spp. and *Plasmodium* spp. was found in Passeridae, as revealed by genetic analysis of Cytb DNA sequences. In this revision, following preceding investigations, the overall prevalence of *Haemoproteus* spp. was greater than that of *Plasmodium* spp. discovered by molecular techniques in Iran. Besides, the frequency of published genetic variants (lineages) of Haemoproteus is higher than that of Plasmodium. Our result indicated that the frequency of novel genetic variants Haemoproteus and Plasmodium in published reports was 38% and 12.5% of diagnosed lineages, respectively. Conclusion: The region needs more studies to detect parasites in unexplored hosts and vectors, as there is a lack of research. Understanding the prevalence, epidemiology, transmission approach, and vectors of avian blood parasites is crucial for pet health care, zoos, parks, aviaries, and rehabilitation facilities. DOI: https://doi.org/10.60141/AJID/V.2.I.1.7 Keywords: Malaria, PCR, blood parasites, Haemosporidian, Veterinary, Iran

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1. Introduction

Birds host haemosporidian parasites, and a widespread collection of mosquitoes can harbor these protozoa (1-3). Numerous arthropods encompassing vectors of Culicidae, Ceratopogonidae, Hippoboscidae and are considered the foremost source of infection transmitted to birds (1). Plasmodium spp. and Haemoproteus spp. have been reported as malaria malaria-like parasites with substantial and ecological and evolutionary effects on their bird hosts, resulting in mortality and extinction of populations (1, 2, 4, 5). These protozoan parasites are also of exceptional concern to healthcare researchers and veterinarians because of the influence of avian malaria on their fitness and their phylogenetic association with human Plasmodium species. More than 55 species of Plasmodium and 150 species of Haemoproteus can infect an extensive range of avian families (6-12).

Numerous studies have tried to discover different characteristics of avian blood parasite infection in passerine and non-passerine birds by microscopic inspection (13-17), molecular-based techniques (18sr RNA and Cytb genes) (13, 18-22), and histological analysis (23-26) around the world. MalAvi (27), an online database for molecular lineages of avian hemosporidian parasites, can be used for comparative analysis of novel sequences. Various elements are accompanied by avian blood parasite prevalence in their hosts and vectors; ecological and biological factors such as geographical regions and sampling time; the availability of competent vectors (mosquitoes); host immunity strength; and other factors related to host-parasite-vector interactions (19, 28-35).

A significant number of passerine and nonpasserine bird species (more than 548 species) inhabited Iran (36, 37). Despite having a great diversity of bird species here and being focused on the detection of genetic variants of blood parasites and their hematophagous vectors in many parts of the world, the west part of Asia has been insufficiently studied. However, recently, various investigations focused on studying the prevalence and epidemiology of malaria and malaria-like parasites in diverse avian hosts by morphological and molecular approaches in Iran (7, 18–22, 38–50).

2. Materials and Methods

In this regard, the bibliographic review article was designed to deliver a collection of the avian hosts infected by *Plasmodium* spp. and *Haemoproteus* spp. in birds of Iran. Furthermore, the composition of genetically unique and published lineages was reviewed in published reports in PubMed, Google Scholar, and Science Direct until April 2023.

3. Results and discussion

Birds are the most dispersed animals due to their journey throughout the breeding season and migration, which enable them to transfer hematozoan parasites to new geographical areas and intermediate hosts (51, 52). Various investigations were performed for the detection of Plasmodium spp. and related parasite species by microscopic inspection of gametocytes in thin blood smears (18, 38, 39, 41, 44, 47-50, 53, 54), while the other studies accomplished the molecular screening of haemosporidian parasites using amplification of the Cytb gene and DNA sequencing (18-22, 40, 42, 44, 46). In the molecular (Table 1 and Figure 1) and morphological studies (Table 2), the highest frequency of examined species belonged to the Accipitridae and Phasianidae families. respectively.

The comparative result showed that the number of bird species examined by molecular method (n = 115) was also higher than the morphological report (n = 78), indicating more interest in genetic analysis for precise identification of genetic variation in blood parasites (4, 16). The highest number of recorded infections of *Haemoproteus* spp. and *Plasmodium* spp. was found in Passeridae, as revealed by genetic analysis of *Cytb* sequences (Table 2).



Figure 1. Frequency of detected parasites by molecular approaches in birds of Iran

Table 1. Frequency of detection of malaria and malaria-like parasites by molecular approach in birds of Iran. The number of bird species, the number of *Plasmodium* spp. and *Haemorpoteus* spp., and their lineages are given. Novel published lineages are in bold format.

Bird Family	Number of bird species	Plasmodium spp. (n) ²	<i>Plasmodium</i> lineages	Haemoproteus spp. (n) ²	Haemoproteus lineages	References
Acrocephalidae	5	0	_	12	ACDUM1, ACDUM2, ACDUM3, ARW1, ACSTE1, BRW1, GRW03, HIP2, IDPAL01 , HIP4, LULU1, HIICT1	(18)
	5	1	SYBOR10	7	SW4, GRW01, ARW1, MW3, ARW1, MW1, HIP2	(46)
Accipitridae	8	0	-	б	ACCBAD02, BUTBUT04, BUTBUT05, BUTBUT06, CIAE04, MILMIG01	(19)
	14	1	LK05	2	AQUCYR01, MILANS02	(22)
	1	0	-	1	BUTBUT15	(43)
Aegithalidae	1	0	-	2	ACDUM2, AEGCAU05	(18)

Alcedinidae	1	0	-	1	ALCATT01	(18)
Anatidae	5	1	SW5	3	ANACRE03, AYTFER01, AYTFER02	(22)
Columbidae	1	0	-	3	COLIV03, COQUI05, HAECOL1	(20)
	2	0	-	1	HAECOL1	(22)
Corvidae	2	3	SGS01, ROFI04, LINN01	0	-	(20)
Emberizidae	3	0	-	6	EMBUC01, GRBRU01, GRBRU02, GRMEL02, GRMEL01, SISKIN01	(18)
Falconidae	2	0	-	3	FALTIN01, LK03, FALSUB02	(19)
	2	0	-	1	LK03	(22)
Fringillidae	5	0	-	2	CCF6, PYERY01	(18)
r ringinidae	1	1	LK05	0	-	(46)
Hirundinidae	2	0	-	1	HIRUS17	(18)
Hypocoliidae	1	0	-	1	PADOM05	(46)
Laniidae	1	0	-	1	RB1	(46)
Leiothrichidae	1	0	-	1	ARGCAU1	(46)
Motacillidae	1	1	MOALB03	1	YWT3	(18)
Muscicapidae	6	0	-	3	FISEM02, FISEM01, OEOEN01	(18)
	6	4	SGS1, LUME4, SYBOR02, LK05	3	PADOM05, SFC1, ROBIN1	(46)
Nectariniidae	1	0	-	2	CCF2, PAHIS2	(46)
Oriolidae	1	0	-	0	-	(46)
Passeridae	3	1	GRW04	б	PADOM05, PADOM32 , PAHIS2, PYERY01, PETPET02 , PETPET01	(18)
	4	4	SGS1, GRW04, PADOM02, PBPIP1	10	GYMXAN1, GYMXAN2, PAHIS1, PADOM05, PAHIS2, HIP2,	(46)

					PADOM34,	
					PADOM03,	
					PADOM35	
					PAHIS1	
	1	0	-	2	PADOM41	(42)
Paridae	2	0	-	1	PARUS1	(18)
	3	1	ORW1	1	ACDUM1	(18)
Phylloscopidae	2	0	-	3	PAHIS2, PADOM05, WW1	(46)
Picidae	1	0	-	1	SISKIN01	(18)
Pycnonotidae	2	2	LUME4 , GRW04	3	PYCLEU2 , CCF2, BUL1	(46)
Scotocercidae	1	1	LK05	0	-	(46)
Sittidoo	1	0	-	2	PARUS1, SITTEP01	(18)
Sittiuae	1	0	-	2	SITTEP02, SITTEP03	(46)
Strigidae	3	0	-	3	TYTAL04, LK03, OTSCO07	(19)
0	3	0	-	1	STAL06	(22)
Sturnidae	1	0	-	1	LAMPUR01	(18)
	2	0	-	1	CUCUR01	(18)
Sylviidae	6	2	LK05, GRW04	12	SYHOR01, SYLALT01 , CURCUR01, LWT1, PAHIS2, SYAT01, SYAT07, SYNIS2, CWT2, CWT2, CWT3, CWT7, PYCLEU2	(46)
Turdidae	1	1	SYAT5	1	TURDUS2	(18)
Total (n)	115	24 (17.5%)	-	113 (82.4%)	-	-

¹ Number of examined birds' species is given.
 ² Number of detected parasites' lineages by molecular technique in each bird family is given.

Accipitridae Acrocephalidae Acrocephalidae	8 3 2	-	+	(41)
Acrocephalidae Acrocephalidae	3 2	-		1
Acrocephalidae	2		+	(39)
Acrocephalidae		-	+	(38)
Асгосерпаниае	3	-	+	(39)
	2	-	+	(38)
	9	+	+	(50)
Anatidae	NA	+	-	(54)
	NA	+	-	(44)
	1	+	+	(49)
Calanda da a	1	-	+	(48)
Columbidae	1	+	-	(54)
	1	-	+	(44)
East	1	-	+	(39)
Emberizidae	2	-	+	(38)
Falconidae	2	-	+	(41)
Enin cillido o	4	-	+	(39)
Fringlindae	2	-	+	(38)
Hirundinidae	2	-	+	(39)
Laniidae	1	-	-	(38)
Motacillidae	1	-	+	(39)
M · · · 1	2	-	+	(39)
Muscicapidae	3	-	-	(38)
	1	-	-	(39)
Paridae	1	-	-	(38)
	2	-	+	(39)
Passeridae	2	-	+	(38)
	1	+	_	(47)
	1	+	-	(53)
	4	+	+	(49)
Phasianidae	4	+	+	(48)
	2	+	_	(54)
Phylloscopidae	3	-	_	(39)
Sittidae	1	_	+	(38)

Table 2. Occurrence of malaria and malaria-like parasites by morphological inspection in birds of Iran.

Strigidae	3	-	+	(41)
Turdidae	1	-	-	(39)
	1	-	+	(38)
Total (n)	78			

* Number of examined birds' species is given. The positive and negative specimens by morphological inspection are highlighted with (+) and (-) symbols. NA: not applicable.

In this revision, following preceding investigations in different geographical regions, the number of detected lineages of *Haemoproteus* spp. (n = 113, n = 113)82.4%) was greater than *Plasmodium* spp. (n = 24, 17.5%) discovered by molecular techniques in Iran (18, 22, 46). The result, summarized in Table 1, indicated that the frequency of novel genetic lineages of Haemoproteus and Plasmodium in published reports was 38% and 12.5% of diagnosed lineages, respectively. The Haemoproteus lineages of IDPAL01, ACCBAD02, BUTBUT04, BUTBUT05. BUTBUT06, CIAE04, MILMIG01, AQUCYR01, AEGCAU05, ALCATT01, ANACRE03, AYTFER01. AYTFER02, EMBUC01, GRBRU01, GRBRU02, GRMEL02, GRMEL01, FALTIN01, FALSUB02, HIRUS17, ARGCAU1, FISEM02, FISEM01, OEOEN01, PADOM32, PETPET02, PETPET01, GYMXAN1, GYMXAN2, PADOM34, PADOM35, PADOM41, PYCLEU2, SITTEP01, SITTEP02, SITTEP03, OTSCO07, STAL06, SYLALT01, PYCLEU2, and CUCUR01, and Plasmodium lineages of LUME4 and MOALB03 were novel and first records for Iranian birds' hosts, whereas the rest of detected lineages have been reported in preceding studies around the world (Table 1).

The *Plasmodium* lineages of LK05 and GRW04 were repeated and common genetic variants discovered in birds of Iran, which were inconsistent with global records as they were introduced as generalist infections in birds (7). The submitted gene sequences of *Plasmodium* and *Haemoproteus* lineages have 479 base pairs (bp) in length and are accessible in NCBI with the following IDs: MG976505-MG976576 (18), MK929542-MK929552 & MN224223-MN224224 (22), MN224217-MN224231 & MW209702MW209713 (19), MT802174-MT802190 (20), MG428417 (43), KY885015 and KY885016 (42), and MT925798-MT925931 (46).

Though the overall prevalence of Haemoproteus spp. was higher than other genera in more reports (18, 19), in some morphological research, no infection with Plasmodium was detected in any host (38, 39, 41). Furthermore, novel lineages of blood parasites that have not been previously detected around the world were identified in several investigations diagnosed by genetic analysis of DNA sequences (18-20, 22, 46). Although the microscopic method is introduced as the gold standard for the identification of malarial blood parasites (16), the more accurate and sensitive potency of molecular approaches should be used in parallel (21, 22). Haemosporidian parasite infections affect the population structure by causing short-term or continuing reductions in their richness and extinction (55). So, due to the undesirable influence of parasites, detection and surveillance of parasites in domesticated, freeliving, wild birds, and captive birds or pets are essential to protecting birds like raptors that are crucial for keeping balance in trophic levels of the food web (56, 57). Captive birds, especially raptors (Accipitridae, Falconidae, and Strigidae), are susceptible to infectious diseases. Thus, the higher prevalence of captive raptors to hemosporidian parasites might be associated with stressful conditions like captivity, which diminish immune system function to clear infections from their bloodstream (19, 41).

Furthermore, the transmission of parasites will be facilitated by migratory birds in charge of the intercontinental spreading of avian infections on different continents and the reversal track back to their home (44, 58–60). In this regard, shared-

structure populations such as Anatidae have a great chance of harboring infections (58, 61). The surveillance of contagion pathogens in domestic birds (e.g., Phasianidae) and pets is vital due to their potential effects on the meat and egg production of chickens, turkeys, and fowl and their direct exposure to other animals and humans (22, 48, 62, 63). While some domestic species of Phasianidae and other pet families (20, 22) in some studies were not infected with blood parasites, further surveys have to be designed to detect infections in other parts of the country.

4. Conclusion

Due to the limited number of studies in this region, designing further investigations for the detection of parasites in numerous unstudied hosts and vectors will provide valuable data. More understanding of the prevalence and epidemiology of avian blood parasites, transmission approaches, and vectors is compulsory for pet healthcare centers, zoos, parks, and rehabilitation facilities to protect these treasured bird species.

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