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Study of Avian Haematozoa in Baghdad City and Neighboring Areas

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ABSTRACT

Urbanization is always linked to biodiversity loss. Effects of urbanization and population growth on birds and their diseases are not understood well. Only few works carried out in Baghdad city on avian haematozoa. Between September 2019 and August 2020, a total of 254 birds either mist netted or purchased in Baghdad city. Blood smears were examined for haematozoa. The haematozoa prevalence is 15%. It is 15.2% among non-passeriformes birds and 14.8% among Passeriformes hosts. Infection among male hosts is 14.5% while it is 15.5% in females. Male and female non-passeriformes hosts acquired 18.5% and 12.8% infection rates respectively, while male and female Passeriformes acquired 11.9% and 21.1% respectively. Host transformations occurred frequently in the new urban host-parasite complex, but still there is a need for quantification studies of the same avian blood parasite species in urban and rural sites. *Plasmodium* spp. are the most common widely distributed parasite among the avian hosts examined in Baghdad city. Recording of *Haemoproteus alaudae* from *Lullula arborea* constitutes a new host record.

Keywords: Baghdad city, Haematozoa, *Haemoproteus*, *Leucocytozoon*, *Plasmodium*.

Introduction

Iraq's biodiversity had been largely degraded due to the uncontrolled hunting, harvesting and illegal trade in threatened species, the absence of key biodiversity areas protection programs, environmental pollution and undisciplined urban development (Price, 2018).

Understanding the effect of various landscape characteristics on the interactions of parasite-host complexes is of important relevance in a world with fast changes in the environment (Loiseau *et al.*, 2010; Belo *et al.*, 2011). Alteration of natural environments into urban settlements affects the

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abundance and distribution of wildlife species, so it can be the main driver to change the dynamics of the parasite ecology (Bradley and Altizer, 2007; Hernandez-Lara *et al.*, 2017).
Urbanization is always linked to biodiversity loss in creating modulated new environments that only few species could survive (McKinney, 2002; Sol *et al.*, 2014; Seress and Liker, 2015). Grimm *et al*. (2000) and Bradley and Altizer (2007) found that the transformation of the landscape as a result of urbanization implies wide environmental effects that surpass cities' geographic boundaries.

Effects of urbanization and the growth of the urban human population on birds and their diseases are still underestimated (Sehgal, 2015; Bichet *et al.*, 2020).
Are the blood parasites that infect birds living in a city are affected by the growing urbanization? Some researchers found differences in infection according to habitat type and urbanization degree (Belo *et al.* 2011; Hernandez-Lara *et al.*, 2017; Bentz *et al.*, 2006; Geue and Partecke, 2008; Horak *et al.*, 2001; Okanga *et al.*, 2013; Carbo-Ramirez *et al.*, 2017; Santiago-Alarcon *et al.*, 2020), while Fokidis *et al.* (2008) and Bichet *et al.* (2020) found no differences in the prevalence of avian haematozoa between rural and urban habitats.

There are about 10000 bird species in the world. They act as pollinators, control pests, integral sources of food, and fertilizers (Herzog *et al.*, 2016). They are of extreme ecological importance (Tabur and Ayvaz 2015) but infected with many intracellular blood parasites such as *Haemoproteus*, *Plasmodium*, *Leucocytozoon* etc. (Quillfeldt *et al.*, 2011). These parasites could cause high mortality, reproduction decline, and coloration change of plumage (Soulsby, 1982; Quillfeldt *et al.*, 2011; Elahi *et al.*, 2014). The dipteran vectors of blood parasites are Culicidae mosquitoes with 54 species recorded in Iraq (Rueda *et al.*, 2008; Kim *et al.*, 2009; Santiago-Alarcon *et al.*, 2012) for *Plasmodium* transmission, Ceratopogonidae biting midges with 24 species (Alwin-Kownacka *et al.*, 2016; Nourani *et al.*, 2020) for *Haemoproteus* transmission, and Simuliidae black flies with 14 species (Coleman *et al.*, 2006; Murdock *et al.*, 2015) as *Leucocytozoon* vectors (Hasson, 2015; Van Hemert *et al.*, 2019).

Only few works on avian haematozoa carried out in and around Baghdad city. These include Mohammad and Al-Moussawi (2012) who examined 190 passerine birds. Then Mohammad and

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Al-Moussawi (2013) studied the haematozoa of resident urban birds in three cities in Iraq including Baghdad in which they examined 6 bird species. Al-Biatee (2014) detected some protozoal infections in domestic quails in Baghdad city. Jassim (2016) examined 13 individuals of the collared dove. Finally, Jassim and Hadi (2018) examined 20 wild quails.

The aim of the present study is to survey, detect, and identify the parasite species distribution among the avian hosts in the Baghdad area.

Materials and methods

Study area: the study area is in Baghdad area, central Iraq (E 33.34058, N 44.40088) (fig. 1). The study area is situated within Middle East Steppe Ecoregion PA0812 which is considered vulnerable in Iraq (WWF, 2009).

Climate: Annual rainfalls range between 100-200 mm with nearly all of it falling between November and April. Annual rainfall experienced variable changes from 1951–2000 (World Bank, 2017). According to 39, the climate of Baghdad is a hot desert with cool to mild, wet, short winters and intense dry, hot, prolonged summers. Temperatures in summer nights rarely dropped below 24 °C. The warmest month is July with 25.5°C-44°C on average . The highest temperature degree recorded in Baghdad was 51.8 °C on 28 July 2020 (Cappucci and Salim, 2021). The coldest month is January with 3.8°C- 15.5°C on average and dropping of temperature below freezing occurs twice/year. The months with rainfall in the Baghdad area are January to May and October to December. For the last 100 years, on January 11th, 2008 light snow fell on Baghdad. Another snowfall with relatively more accumulations was on February 11th, 2020.

Sampling: The present work was conducted according to the national and institutional guidelines for animal use and care. A total of 254 birds comprises of 26 species were examined between September 2019 to August 2020. Sixteen of them are non-passeriformes and 10 are Passeriformes (table 1). Fourteen were residents, two spring and autumn visitors, two winter visitors, and one passage migrant (table 2).

The mist nets were used to capture birds from early morning until noon. Some birds were purchased from the local markets. The blood was drained from the brachial vein. The smears air

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dried, rinsed in absolute ethanol and finally stained with Giemsa's stain at pH 7.2 and diluted to 1:10 for one hour (Santiago-Alarcon and Carbó-Ramírez, 2015). The examined birds were freed at their capture site while the purchased ones were released out through the windows of the laboratory in Uruk University. The domestic chickens were examined at the homes and farms of the families who raised them. Determining the residence status of birds was done following (Salim *et al.*, 2006; Pope and Zogaris, 2012). Eighteen bird species are resident including 3 introduced species, 3 winter visitors, 3 passage migrants, and two spring and autumn visitors (Table 2). The identification of parasites were determined following (Forrester *et al.*, 1977; Valkiūnas, 2005).

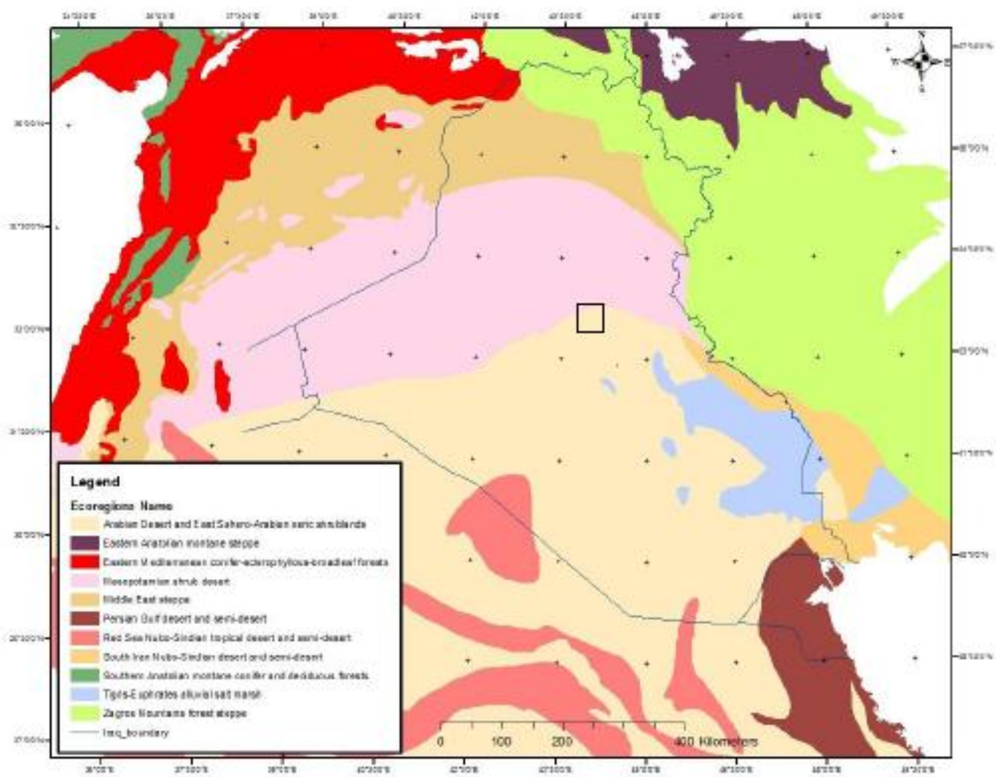

Figure 1. Map of Iraq showing the ecoregions of Iraq and the location of the study area at Baghdad city. From: Abdulhassan *et al.* (2011).

Statistical analysis: The results are analyzed with the SPSS ver. 17 using Pearson Chi Square Test. P value > 0.05 is considered statistically significant.

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Results

Tables 1&2 show that the prevalence of haematozoa among the examined birds is 15%. It is 15.2% among non-passeriformes birds and 14.8% among Passeriformes hosts. Infection among male hosts is 14.5% while it is 15.5% in females.

Table 1 shows that male and female non-passeriformes hosts acquired 18.5% and 12.8% infection rates respectively. In contrast, the male and female Passeriformes acquired 11.9% and 21.1% infection rates respectively.

Table 1. Sex/no. of examined and infected birds among Passeriformes and non-Passeriformes hosts

Sex/no. of examined and infected birds	♂		%	♀		%	% Overall infection
	Number examined	Number infected		Number examined	Number infected		
Non-Passeriformes	54	10	18.5	78	10	12.8	15.2
Passeriformes	84	10	11.9	38	8	21.1	14.8

In some instances, double infections of *Leucocytozoon* sp. and *Plasmodium* sp. were observed. Statistical analysis showed no significant differences between the infection in male and female hosts and the distribution of parasites in Passeriformes and Non-passeriformes birds as Pearson Chi Square was 0.732.

The number of parasite species is 9 in three genera. The most prevalent genus is *Haemoproteus* with 5 species namely, *Haemoproteus alaudae* (fig.2), *H. columbae* (fig.3), *H. turtur* (fig.4), *H. lanii* and *H. passeris*.

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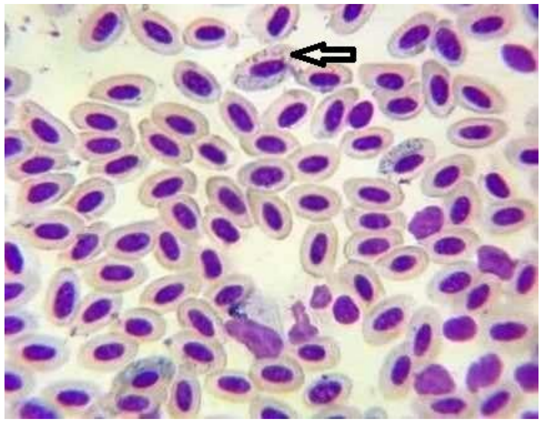

Figure 2. *Haemoproteus alaudae* in a blood smear of the woodlark *Lullula arborea.* The arrow indicates presence of two haemoproteids within the same erythrocyte.

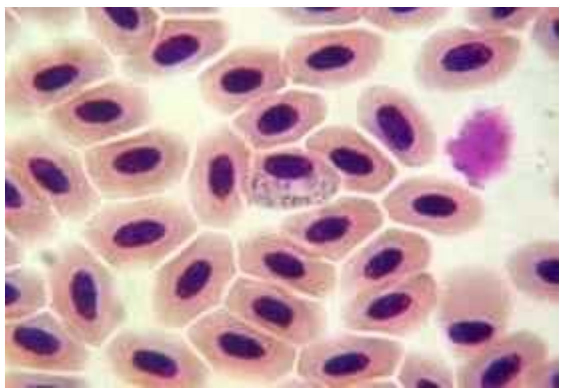

Figure 3. *Haemoproteus columbae* from the domestic rock pigeon *Columba livia domestica.*

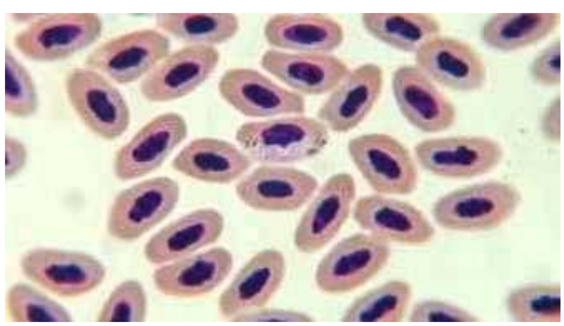

Figure 4. *Haemoproteus turtur* from domestic rock pigeon *Columba livia domestica.*

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Table 2. Summary of avian haematozoa survey results in Baghdad city and adjacent areas including bird species, family and order, number of examined birds, number of infected birds, percent infection rate, bird migration status, blood parasite species and remarks.

Order	Family	Bird scientific name	No. exam.		No. infected		% infection			Migration status	Parasite name	Remarks
			♂	♀	♂	♀	♂	♀	total			
Anseriformes	Anatidae	Marmaronetta angustirostris (Menetries, 1832)	3	3	0	1	0	33.3	16.7	R	*Plasmodium relictum*	
Charadriiformes	Recurvirostridae	*Himantopus himantopus* (Linnaeus, 1758)	2	2	1	0	50	0	25	R	*Plasmodium relictum*	
		Recurvirostra avosetta Linnaeus, 1758	2	1	0	1	0	50	33.3	R	*Plasmodium relictum*	
Galliformes	Phasianidae	*Gallus gallus domesticus* (Linnaeus, 1758)	15	15	1	0	66.7	0	66.7	R	*Plasmodium* sp.	local race chicken
		Coturnix coturnix (Linnaeus, 1758)	1	1	0	1	0	50	50	PM	*Plasmodium* sp.	

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		Coturnix japonica Temminck & Schlegel, 1848	1	11	0	1	0	9.1	9.1	R Introduced	*Plasmodium relictum*	
Columbiformes	Columbidae	Columba livia domestica Gmelin, 1789	20	15	3	2	15	13.3	14.3	R	*Haemoproteus columbae**, *Haemoproteus turtur*, *Plasmodium relictum***, *Lecocytozoon marchouxi*** , *Leucocytozoon* sp.	*Heavy to Moderate infection **Double parasitic infection in the same individual. All individuals are from domestic race raised in houses.
		Columba oenas Linnaeus, 1758	2	1	1	0	33.3	0	33.3	R	*Haemoproteus columbae*	
		Columba palumbus Linnaeus, 1758	5	3	1	1	12.5	12.5	25	R	*Haemoproteus columbae*	

		Spilopelia senegalensis (Linnaeus, 1766)	6	7	2	1	33.3	14.2	23.1	R	*Haemoproteus columbae, Haemoproteus turtur*	
		Streptopelia decaocto (Frivaldszky, 1838)	5	5	1	1	20	20	20	R	*Lecocytozoon* sp.+ *Plasmodium relictum***	**Double parasitic infection in the same individual.
		Streptopelia turtur (Linnaeus, 1758)	1	2	0	1	0	50	33.3	R	*Plasmodium* sp.	A summer visitor bird.
Passeriformes	Alaudidae	*Galerida cristata* (Linnaeus, 1758)	3	5	1	1	33.3	20	25	R	*Haemoproteus alaudae*	Very common resident bird.
		Lullula arborea (Linnaeus, 1758)	3	0	1	0	33.3	0	33.3	WV	*Haemoproteus alaudae*	A winter visitor bird. Some erythrocytes have a double infection with the parasite.

	Laniidae	*Lanius isabellinus* Hemprich & Ehrenberg, 1833	4	1	1	0	25	0	20	SAV	*Haemoproteus lanii*	
		Lanius nubicus Lichtenstein, 1823	3	0	1	0	33.3	0	33.3	SAV	*Plasmodium relictum*	
	Ploceidae	Passer domesticus (Linnaeus, 1758)	12	12	3	4	25	33.3	29.2	R	*Leucocytozoon* sp., *Haemoproteus passeris*, *Plasmodium relictum*	
	Pycnonotidae	Pycnonoyus leucogenys (Gray, JE, 1835)	10	7	1	1	10	14.3	11.8	R	*Leucocytozoon* sp.	
	Sturnidae	*Sturnus vulgaris* Linnaeus, 1758	13	11	2	2	15.4	18.2	16.7	WV	*Plasmodium relictum*, *Leucocytozoon* sp.*,	*The parasite intensity is very low.

Negative birds: scientific name (number): *Lanius isabellinus* (PM, 2♂, 1♀); *Lanius nubicus* (PM, 2♂, 1♀); *Meleagris gallopavo* (R, introduced, 6♂, 3♀); *Numida meleagris* (R, introduced, 6♂, 5♀); *Passer moabiticus* (R, 2♂); *Pterocles alchata* (R, 4♂, 2♀); *Spatula clypeata* (WV, 3♂, 2♀).

Migration status: R=Resident, PM=Passage migrant, SAV= Spring and autumn visitor, SPM=Summer passage migrant, WV=Winter visitor.

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Discussion

Present study differs from the previous studies carried out in Baghdad city. It differs from Mohammad and Al-Moussawi (2012) in that they confined their study to passerine birds only. It also differs from Mohammad and Al-Moussawi (2013) in that they studied three cities in the north, middle, and south of Iraq and not devoted to Baghdad city. The works of Al-Biatee (2014) and Jassim and Hadi (2018) deal with one bird species, the domestic and wild quail respectively.

The prevalence of haematozoa infects birds in Baghdad city is (15%). It is far different from that of Mohammad and Al-Moussawi (2012) who found that 10.5% of wild birds in Baghdad city. This could be related to the material used in this study which includes many examples of domestic birds that are raised within or around houses in relatively large numbers which makes them more exposed to potential vectors of haematozoa. Tables 1&2 also show that the non-passerine birds have a slightly more prevalence of blood parasites than passerine birds. This is could be correlated to the infection of a large part of examined birds that are raised in large numbers and crowded in a small place like the domestic pigeon *C. l. domestica* and *G. gallus domesticus* makes them more exposed to vectors. On the other hand, all of the passerine birds are wild and some of them are only juveniles of the same season and hence there is no enough time available for them to acquire infections.

All of the five *Haemoproteus* species were previously reported from Iraq. *Haemoproteus alaudae* (fig. 2) is confined to Alaudidae members and widely distributed in the regions of hosts existence range. Mohammad (1990) recorded it from Iraq in *Alaemon alaudipes* (Desfontaines, 1789), *Ammomanes deserti* (Lichtenstein, 1823), *Eremophila alpestris* (Linnaeus, 1758), *Galerida cristata* and *Melanocorypha calandra* (Linnaeus, 1766). Another 7 lark species were recorded to harbor this parasite in Kenya, Africa (Bennett and Peirce, 1990a). In the present study, *Galrida cristata* and *Lullula arborea* encounter this parasite. Both hosts were mist netted at the outer circumference of Baghdad city. To the best of the knowledge of the authors, this is the first time that *H. alaudae* is recorded from *Lullula arborea* and this should represents a new host record. In some cases two haemoproteids were observed encircling the *L. arborea* erythrocyte nucleus. This may indicates high vector potentiality.

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Haemoproteus columbae (fig. 3) is widely distributed among columbid hosts in this study. However, its absence in two species belonging to genus *Streptopelia* in spite of examining 13 individuals, suggests a host transformation within Baghdad city urban environment. In this respect, Garcia-Longoria *et al.* (2019) noted that the prevalence of avian blood parasite communities is determined by various environmental and geographic factors. It is well known these two birds are very common within human settlements within Baghdad city. Shamsuddin and Mohammad (1980) found it in *S. decaocto* in Badra vicinity, Wasit province in rural eastern parts at the Iranian-Iraqi border, while Jassim (2016) could not find this parasite in *S. decaocto* examined specimens. Mohammad and Al-Moussawi (2013) found it with wide geographical distribution in Iraq. Al-Rubaie *et al.* (2020) found it in *C. l. domestica* in Baghdad city. Shamsuddin and Mohammad (1980) examined *Columba livia, C. palumbus, Streptopelia decaocto* and *S. turtur* collected mostly in rural areas along the eastern strip border with Iran and found it in all of them. The existence of *H. columbae* in Mosul city at the north of Iraq was confirmed by Al-Janabi *et al.* (1980) and Hasan *et al.* (2018) in *C. livia.*
Valkiunas (2005) considered *Haemoproteus turtur* similar to *H. columbae* and *H. palumbis* stating that it represents an intermediate between them. Bennett and Peirce (1990b) decided the synonymity of *H. turtur* to *H. columbae.* Mohammad and Al-Moussawi (2013) found *H. turtur* in *C. livia* and *S. senegalensis* at Baghdad only although they examined the same species in Sulaimaniya and Diwaniya in the north and south of Iraq respectively. This is an indication for a proposed change in host-vector interrelationship at Baghdad urban areas. *Haemoproteus turtur* (fig. 4) is considered in this study as a separate valid species based on the capability of differentiation from *H. columbae* depending on gametocyte morphology and the number, size and distribution of pigment granules.

Haemoproteus passeris is reported once in this study from *Passer domesticus*. It was recorded earlier in the Baghdad area from the same host by (Mohammad, 1990). It is widely distributed throughout the globe except for the Antarctic. 45 listed 15 bird species of the families Passeridae and Ploceidae known to host this parasite including the House Sparrow. These two families are sometimes considered as subfamilies of Ploceidae (Britannica, 2021).

Haemoproteus lanii was reported by Shamsuddin and Mohammad (1980) from *Lanius collurio* Linnaeus, 1758 at Baghdad (central Iraq), Khaniqin and Zurbatiya (Iraq-Iran borderline), *Lanius*

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senator Linnaeus, 1758 at Zawita (north of Iraq), and *Lanius nubicus* Lichtenstein, 1823 at Baghdad, then it was recorded by (Mohammad and Al-Moussawi, 2012) from *L. nubicus*. It could be noted that it is widely distributed among the shrikes family Laniidae and with a wide range of environments.

Plasmodium relictum is considered pathogenic for the wild hosts and is very common with wide distribution among bird species and orders, vector hosts and geographical existence (Valkiūnas, 2005). It was reported globally from 411 bird species belong to 70 families (Atkinson, 2008; CABI. 2021). Reporting of *P. relictum* from rather a wide range of avian hosts in this study indicates a high vector potentiality in the studied area. Martinez-de la Puente *et al.* (2016) found that birds are the most common blood source for *Culex pipiens* which is responsible for the transmission of *Plasmodium*. Furthermore, González *et al.* (2020) found that *C. pipiens,* in spite of human and dog availability, preferred to feed on birds. Rueda *et al.* (2008) and Hantosh *et al.* (2012) described a wide distribution of this mosquito species throughout Iraq. The urban settlements could offer many microenvironments suitable for mosquito breeding providing water, the suitable temperature almost around the year and shaded places. In this context, Wilke *et al.* (2019) concluded that processes of urbanization resulted in creating more ideal habitats for the mosquitoes with fewer predators and more humans, the potential food source. Thus the relatively high *Plasmodium* parasitemia and wide distribution among various avian hosts examined in this study could be explained in view of the previous finding and the intimate mosquito-bird host relationship at the urban habitats. In Iraq it was found in *Calandarella rufiscense, Turdoides altirostris, T. caudatus, Pycnonotus leucogenyes, Phoenicurus phoenicurus, Passer domesticus* (Mohammad and Al-Moussawi, 2012) and *Anas platyrhynchos* (Mohammad, 2016). Some records in this study and that of Mohammad and Al-Moussawi (2012) and Mohammad (2016) are only identified to generic level and this could be related mostly to inadequate staining, poor slide preparation and /or absence of the mature gametocytes that could enable firm identification.

Leucocytozoon marchouxi is one of leucocytozoids species which parasitize Columbiformes birds. The present specimens of *L. marchouxi* are of typical morphology as described by (Valkiūnas, 2005). The gametocytes grow in rounded lymphocytes and monocytes of the bird

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hosts. This parasite species was formerly seen in *C. livia* and *S. decaocto* columbidae birds in Baghdad city (Shamsuddin and Mohammad, 1980; Mohammad and Al-Moussawi, 2012). The other infections of *Leucocytozoon* of this study are referred to *Leucocytozoon* sp. either due to their immature stage of development or to the poor staining and/or preparation.

On the other hand, the migratory birds are 5 species belonging to Galliformes (1 species) and Passeriformes (4 species). The only non-passerine migrant bird examined in this study is a female *Coturnix coturnix* which is a passage migrant throughout southern and central Iraq (Salim *et al.*, 2006, 2020) infected with *Plasmodium* sp. As this genus of haematozoa is of worldwide distribution in addition to the short time spent in Baghdad area, it is more likely that it was infected before reaching here. The other 4 bird species are passerines. The two shrikes, *Lanius isabellinus* and *L. nubicus* are spring and autumn visitors spending only a short time in the Baghdad area during their migration route. They were found infected with *Haemoproteus lanii* and *Plasmodium relictum* respectively. The fully mature gametocytes observed in them allow to assume that the infection occurred in winter or summer habitats. The woodlark *Lullula arborea* and starling *Sturnus vulgaris* are winter visitors in Iraq. The arthropod vectors of *H. alaudae, P. relictum* and *Leucocytozoon* sp. (table 2) are inactive during winter cold months, so it is also possible that the birds arrive to our area in winter with their parasites. In contrast, all resident birds acquired their infections locally in the Baghdad area since they did not experience local migrations.

Conclusions

In comparing the present results with the available comparable Iraqi literature it could be concluded that host transformations appear to have occurred frequently in the new urban host-parasite copmlex, except for the recording of *H. alaudae* from *G. cristata*. The latter species is seldom seen touching human settlements around Baghdad city.

Through reviewing Iraqi literature on avian haematozoa in urban and suburban areas, it is clear that there is a need for quantification studies of the same avian blood parasite species in urban and rural sites. This is proposed to clarify the blurred situation of only recording the incidence of the same parasite in the same host at urban and rural sites.

Recording of *Haemoproteus alaudae* from the woodlark *Lullula arborea* constitutes a new host record for the parasite.

Plasmodium spp. are the most common widely distributed among the avian hosts examined in Baghdad city indicating high parasite adaptability and vector potentiality.

Conflict of interest statement

The authors declare that they have no conflict of interests.

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