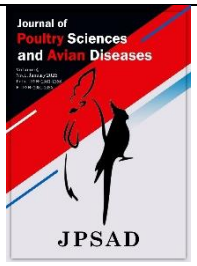
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Haemoparasites and ectoparasites in raptors admitted to a wildlife rehabilitation center in the Mashhad area, Khorasan Razavi province: Frequency and literature overview of haemoparasites in Northeastern Iran

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A B S T R A C T

Haemoparasites and ectoparasites are prevalent in raptors and can lead to illness or death. This study assessed the frequency of blood and external parasites in raptors at a wildlife rehabilitation center in the Mashhad area using microscopic examination. Between 2023 and 2024, 51 blood samples were collected from 11 golden eagles (*Aquila chrysaetos*), 3 Eurasian sparrowhawks (*Accipiter nisus*), 28 common buzzards (*Buteo buteo*), 8 common kestrels (*Falco tinnunculus*), and 1 long-eared owl (*Asio otus*). Thin blood smears were prepared for analysis, and all birds were examined for ectoparasites. Haemoparasites and ectoparasites were only found in golden eagles and common buzzards. Among common buzzards, 2 of 28 (7.14%) were infected with *Leucocytozoon* spp., and another 2 of 28 (7.14%) with *Haemoproteus* spp. In golden eagles, 3 of 11 (27.27%) were infected with *Leucocytozoon* spp. *Laemobothrion maximum* (chewing louse) was identified in 8 of 28 (28.57%) common buzzards, and larval *Argas* specimens morphologically compatible with *A. persicus* were found in 2 of 11 (18.18%) golden eagles. In summary, *Leucocytozoon* spp., *Haemoproteus* spp., *L. maximum*, and larval *Argas* specimens compatible with *A. persicus* were detected in raptors admitted to the Mashhad rehabilitation center.

Keywords: *Raptor, Haemoparasites, Ectoparasite, Mashhad Area.*

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1 Introduction

Raptors play a crucial role in maintaining ecological balance, yet research on their parasites is limited compared to studies on other birds, such as passerines (Clark et al., 2009). Blood parasites, including Leucocytozoon spp., Plasmodium spp., and Haemoproteus spp., are widespread and transmitted by blood-sucking vectors (Valkiūnas, 2005). While often considered harmless, these parasites can cause reduced flight performance, lethargy, weight loss, anemia, air sac inflammation, and arthritis in birds (Remple, 2004). Raptors also host diverse ectoparasites, such as lice, mites, fleas, flies, and mosquitoes, which may cause feather damage, skin irritation, and disease transmission. Severe infestations, especially by lice and feather mites, can significantly harm skin and feathers (De Oliveira et al., 2011). Blood parasites in raptors are diagnosed using microscopic and molecular methods; although molecular techniques are more sensitive, microscopy remains the standard (Valkiūnas, 2005). Ectoparasites are microscopically identified by comparing their morphology to standard keys (De Oliveira et al., 2011). Khorasan Razavi Province in northeastern Iran supports diverse wildlife and many protected areas for raptors. However, few studies have examined blood and external parasite infections in Iranian raptors. The aim of this study was to assess the occurrence and rates of haemoparasite and ectoparasite infections in raptors at a Mashhad-area rehabilitation center and to identify the specific parasites present.

2 Materials and Methods

From December 2023 to July 13, 2024, blood samples and ectoparasites were collected from 51 raptors representing five species admitted to the Mashhad wildlife rehabilitation center (36°18.936′N, 59°34.08′E). None of the raptors had received prior treatment for blood or external parasites. To minimize stress, birds were gently removed from their cages with their heads covered. Blood samples (0.5 mL) were drawn from the brachial vein using a 28-gauge insulin syringe (Swayne et al., 2013), and a thin blood smear was immediately prepared for each bird. Each bird underwent a thorough 20-minute inspection of the head, trunk, wings, tail, and legs. Ectoparasites were collected by hand or tweezers and placed in labeled vials containing 70% alcohol, then sent to the parasitology laboratory. Following Soulsby's methodology, blood smears were fixed with methanol for 1–3 minutes, stained with Giemsa diluted in phosphate buffer (pH=6.8–7.2) at a 1:10 ratio for 30 minutes (Soulsby, 1982), and examined under a 100×microscope lens with oil immersion for haemoparasite identification.

The characteristics used to separate *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* on a blood smear include the presence or absence of pigment granules in erythrocytic parasites, the size and shape of gametocytes, and the absence of asexual schizogony in blood cells. Plasmodium spp. and Haemoproteus spp. produce the pigment granules. but not *Leucocytozoon*. The morphology of *Haemoproteus* gametocytes within an erythrocyte is characterized by a thick, sausage-like and halter-shaped appearance, distinguishing it from *Plasmodium* (Soulsby, 1982).

Ectoparasites from each bird were placed in a tube with 10% potassium hydroxide and boiled until the chitin dissolved. Samples were then rinsed in water for 30 to 60 minutes to remove residual KOH. Dehydration was performed in 70% alcohol for 30 minutes, followed by 90% alcohol for 20 minutes and absolute alcohol for 15 minutes. Cleared ectoparasites were mounted on glass slides with Dibutyl Phthalate Xylene and examined under a binocular light microscope using identification keys (Soulsby, 1982).

Laemobothrion maximum is the largest known bird louse. The head was distinctly flattened, with a very large oral opening extending posteriorly up to the antennal base. The antennal capsules were bulbous and opened ventrally. (Azizi et al., 2013). The taxonomic dichotomous key for *A. persicus* has been detailed through the following characteristics: the body of *A. persicus* is dorsoventrally flattened, and the margin of the body features a lateral suture. This suture consists of rectangular plates on both the dorsal and ventral aspects. The integumentary structure is complex, and there are no eyes present; each tarsus has a pair of claws. The mouthparts are small and located ventrally, consisting of a central toothed hypostome and a pair of palps. In females, the genital cleft is a broad horizontal fissure, while in males, it is in the same location but approximately half the width of the female's and more oval in shape. The stigma is cone-shaped and situated above the coxae of legs three and four. Hall's organ is a complex sensory organ found in soft ticks that serves a chemosensory function distinct from that of insects. (Kohls et al., 1970; Walker et al., 2003). Larvae have a dorsal plate, three pairs of legs, and dorsally located mouthparts. The capitulum is located near the anterior margin of the ventral side. (Walker et al., 2003).

Statistical analysis:

Data were analyzed using SPSS version 22 (Chicago, USA). Confidence intervals of proportions were calculated by the modified Wald method.

3 Results and Discussion

The findings indicated that, among 51 raptors, 13.7% (7 birds) were infected with avian blood parasites. The infected samples were exclusively from two raptor species. The golden eagle exhibited the highest detection frequency at 3/11 (27.27%; 95% CI: 9.20-57.11%), followed by the common buzzard at 4/28 (14.28%; 95% CI: 5.02-32.11%). Only the common buzzard showed *Haemoproteus* spp. infection, recorded at 2/28 (7.14%; 95% CI: 0.90-23.73%) (Fig. 1). *Leucocytozoon* spp. was deteAmong 51 raptors examined, 13.7% (7 birds) were infected with avian blood parasites, limited to two species. Golden eagles had the highest detection rate at 3 of 11 (27.27%; 95% CI: 9.20–57.11%), followed by common buzzards at 4 of 28 (14.28%; 95% CI: 5.02–32.11%). Haemoproteus spp. infection was found only in common buzzards (2 of 28; 7.14%; 95% CI: 0.90–23.73%) (Fig. 1).

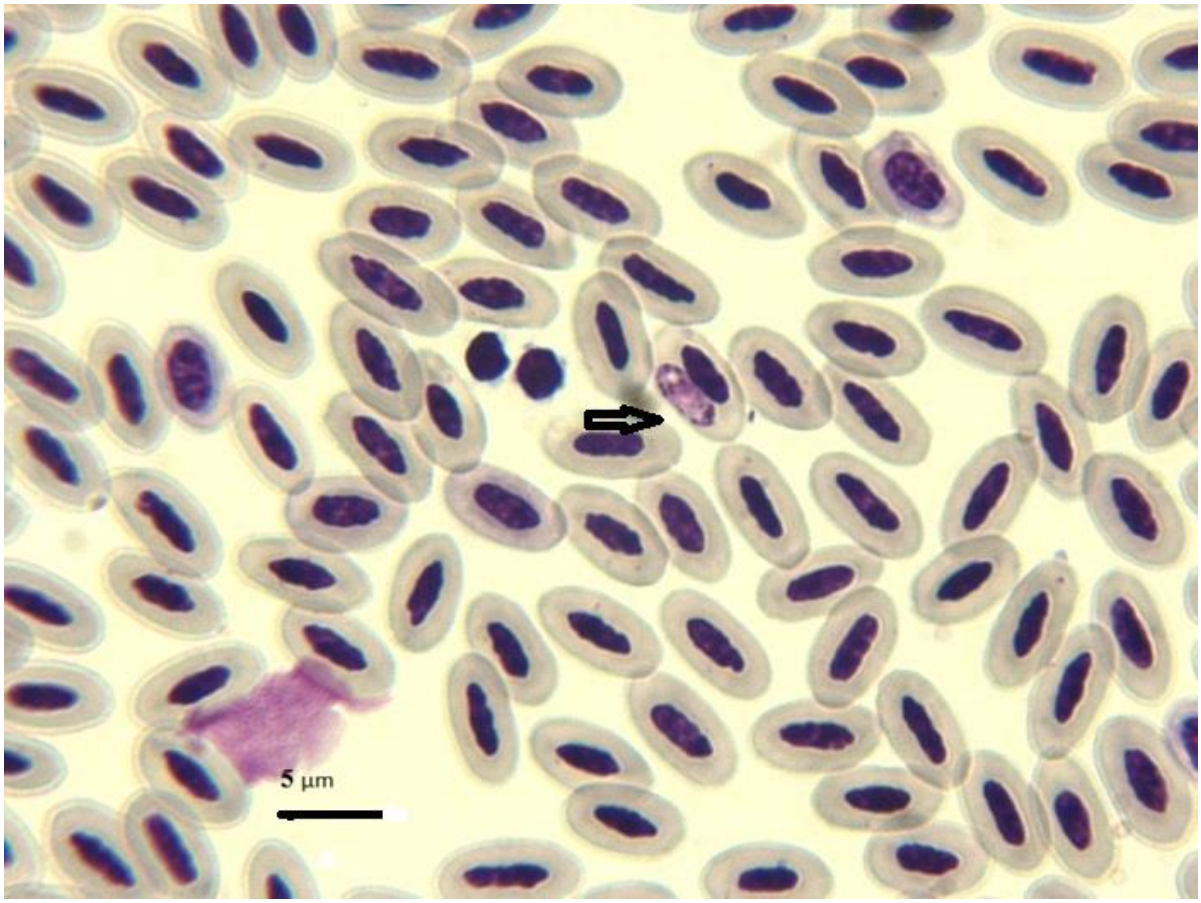


Figure 1. *Haemoproteus* spp. infection in a peripheral blood smear from a common buzzard (*Buteo buteo*). Giemsa stain. Arrows indicate parasitized erythrocytes. Scale bar=5 µm. (×1000 magnification).

Leucocytozoon spp. was detected in 3 of 11 golden eagles (27.27%; 95% CI: 9.20–57.11%) (Fig. 2) and 2 of 28 common buzzards (7.14%; 95% CI: 0.90–23.73%) (Table 1). No Plasmodium infection was observed. Ectoparasites were found in 10 of 51 raptors (19.6%). Adult *L. maximum* (chewing lice) was present in 8 of 28 (28.57%; 95% CI: 15.07–47.24%) common buzzards. We found larval Argas specimens morphologically compatible with A. persicus in 2 of 11 (18.18%; 95% CI: 3.99–48.85%) golden eagles. A few adult *A. persicus* ticks were also found in the holding area (Figs. 3–5). No other raptors were infested with ectoparasites (Table 2), and no bird had concurrent haemoparasite and ectoparasite infections. However, the infection frequencies determined by microscopic examination may underestimate the actual occurrence of haemoparasite infections because of the limited sensitivity of this diagnostic method. Furthermore, given the small sample sizes of common kestrels, Eurasian sparrowhawks, and long-eared owls, haemoparasite infections in these species cannot be ruled out.

Table 1. Frequency of haemoparasite infection in raptors at the rehabilitation facilities of the Khorasan Environmental Department.

Bird	*Leucocytozoon spp.*		*Haemoproteus spp.*		Any haemoparasite		Total
	Positive n/N (%)	Negative	Positive n/N (%)	Negative	Positive n/N (%)	Negative	
Golden eagle (Aquila chrysaetos)	3/11 (27.27)	8	0	11	3/11 (27.27)	8	11
Common buzzard (Buteo buteo)	2/28 (7.14)	26	2/28 (7.14)	26	4/28 (14.28)	24	28
Common kestrel (Falco tinnunculus)	0	8	0	8	0	8	8
Eurasian sparrowhawk (Accipiter nisus)	0	3	0	3	0	3	3
Long-eared owl (Asio otus)	0	1	0	1	0	1	1
Total	**5/51 (9.80)**	**46**	**2/51 (3.92)**	**49**	**7/51 (13.7)**	**44**	**51**

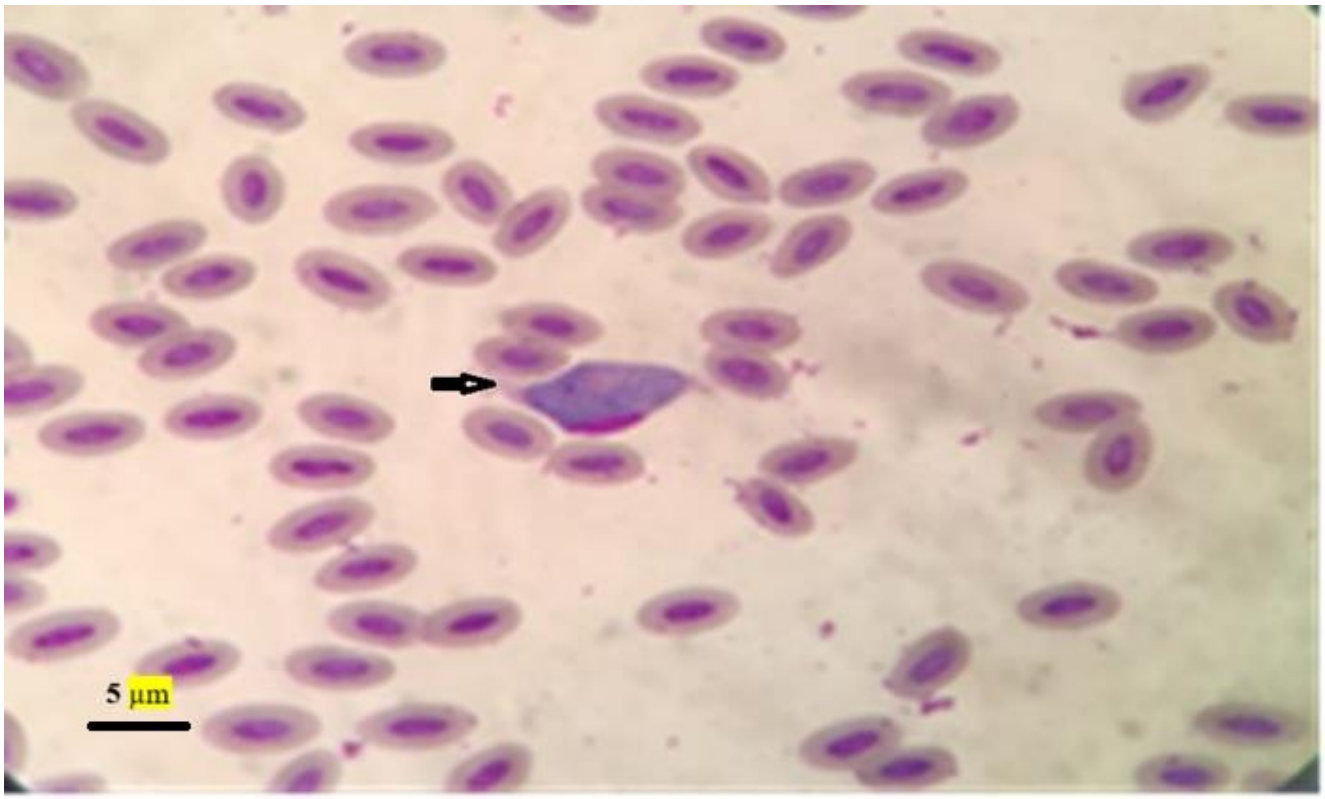


Figure 2. *Leucocytozoon* spp. infection in a peripheral blood smear from a Common buzzard (*Buteo buteo*). Giemsa stain. Arrows indicate parasitized erythrocytes. Scale bar=5 µm (×1000 magnification).



Figure 3. *Laemobothrion maximum* (size=8-11 mm), common buzzard. Scale bar=1 mm

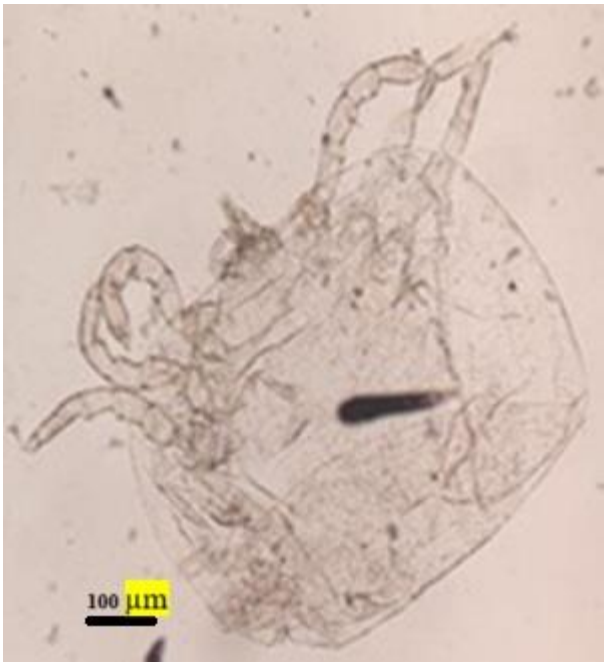


Figure 4. Larval *Argas* specimen morphologically compatible with *A. persicus* (size=500-700 µm) collected from a golden eagle. Scale bar=100 µm.



Figure 5. The adult *Argas persicus* (6-9 mm) in the holding area. Scale bar=1 mm

Table 2. Frequency of ectoparasite detection in birds of prey kept at the rehabilitation center of the Khorasan Environmental Department.

Bird	*Argas persicus*		*Laemobothrion maximum*		Any ectoparasite		Total
	Positive n/N (%)	Negative	Positive n/N (%)	Negative	Positive n/N (%)	Negative	
Golden eagle (Aquila chrysaetos)	2/11 (18.18)	9	0	11	2/11 (18.18)	9	11
Common buzzard (Buteo buteo)	0	28	8/28 (28.57)	20	8/28 (28.57)	20	28
Common kestrel (Falco tinnunculus)	0	8	0	8	0	8	8
Eurasian sparrowhawk (Accipiter nisus)	0	3	0	3	0	3	3
Long-eared owl (Asio otus)	0	1	0	1	0	1	1
Total	**2/51 (3.92)**	**49**	**8/51 (15.68)**	**43**	**10/51 (19.6)**	**41**	**51**

Various studies in Iran have identified infections with *Haemoproteus* spp., *Leucocytozoon* spp., and *Plasmodium* spp. in various raptor species using microscopy and molecular techniques (Table 3). Among these studies, Razmyar et al. (2016) reported infections of *Leucocytozoon* spp. in two golden eagles, one common buzzard, and one eagle owl at rehabilitation centers in the Mashhad region, utilizing both microscopy and molecular techniques (Razmyar et al., 2016). Their findings indicated that approximately 4/30 (11%) of the raptors tested positive for

this parasite. This result aligns closely with the outcomes of the current research. However, their study did not reveal any infections with *Haemoproteus* spp. (Razmyar et al., 2016). Haemoparasites have been documented in different raptor species in neighboring and other countries (Table 4). Haemoparasite infections in common buzzards have also been reported in Turkey (Zerek et al., 2023), Greece (Markakis et al., 2025), and Bosnia and Herzegovina (Harl et al., 2022). The observed frequency was numerically comparable with that reported in some previous studies; however, direct comparison should be cautiously interpreted because of differences in host composition, sample size, sampling setting, and diagnostic methodology.

Table 3. Studies reporting the frequency of haemoparasite detection in raptors in different provinces of Iran

Province	Year	Common name	No. examined	n/N (%)						Reference
				Haemoproteus spp.		*Leucocytozoon spp.*		*Plasmodium spp.*		
				Mic	PCR	Mic	PCR	Mic	PCR	
East Azerbaijan	2012	Common buzzard	1	0	0	1/1 (100)	0	0	0	Shirazi et al., 2012
Khorasan Razavi	2016	Golden eagle	7	0	0	2/7 (28.57)	2/7 (28.57)	0	0	Razmyar et al., 2016
		Common buzzard	16	0	0	1/16 (6.25)	1/16 (6.25)	0	0	
		Eagle owl	2	0	0	1/2 (50)	1/2 (50)	0	0	
Tehran	2017	Marsh harrier	3	0	0	3/3 (100)	-	0	0	Rasouli et al., 2017
		Tawny eagle	1	0	0	1/1 (100)	-	0	0	
Lorestan	2021	Common buzzard	11	1/11 (9.09)	0	7/11 (63.63)	0	0	0	Shokrani et al., 2021
Khorasan Razavi	2022	Shikra	8	1/8 (12.5)	1/8 (12.5)	0	0	0	0	Nourani et al., 2022
		Golden eagle	1	0	0	0	0	0	0	
		Tawny eagle	1	0	0	0	0	0	0	
		Greater spotted eagle	3	0	0	0	0	0	0	
		Eurasian buzzard	15	4/15 (26.66)	4/15 (26.66)	0	0	0	0	
		Eurasian hobby	1	1/1 (100)	0	0	0	0	0	
		Little owl	3	0	0	1/3 (33.3)	1/1 (100)	0	0	
		Eurasian eagle owl	1	0	0	1/1 (100)	1/1 (100)	0	0	
		Eurasian scops owl	1	1/1 (100)	1/1 (100)	0	0	0	0	
Golestan	2022	Western marsh harrier	2	1/2 (50)	0	0	0	0	0	Nourani et al., 2022
		Black kite	1	1/1 (100)	1/1 (100)	0	0	0	0	
		Egyptian vulture	1	0	0	0	0	0	0	
		Eurasian buzzard	1	1/1 (100)	1/1 (100)	0	0	0	0	
		Golden eagle	1	0	0	0	0	0	0	
		White-tailed eagle	1	0	0	0	1/1 (100)	0	0	
		Greater spotted eagle	13	0	0	0	0	0	1/13 (7.69)	
East Azarbaijan	2022	Eurasian eagle-owl (Bubo bubo)	1	1/1 (100)	0	1/1 (100)	0	0	0	Shirazi et al., 2022

Table 4. Studies reporting the frequency of haemoparasite detection in raptors in neighbouring and selected countries

Country	Year	Common name	No. examined	n/N (%)						Reference
				Haemoproteus spp.		*Leucocytozoon spp.*		*Plasmodium spp.*		
				Mic	PCR	Mic	PCR	Mic	PCR	
Turkey	2023	Common buzzard	17	4/17 (23.52)	4/17 (23.52)	0	0	0	0	Zerek et al., 2023a
		Lesser spotted eagle	11	2/11 (18.18)	2/11 (18.18)	0	0	0	0	
		Short-toed snake eagle	9	1/9 (11.11)	2/9 (22.22)	0	1/9 (11.11)	0	0	
		Long-legged buzzard	5	1/5 (20)	1/5 (20)	0	0	0	0	
		Black kite	1	1/1 (100)	1/1 (100)	0	0	0	0	
Turkey	2023	Lesser spotted eagle	8	3/8 (37.5)	3/8 (37.5)	0	0	1/8 (12.5)	1/8 (12.5)	Zerek et al., 2023b
		Common buzzard	14	1/14 (7.14)	1/14 (7.14)	0	0	0	0	
		Short-toed snake eagle	12	0	1/12 (8.3)	0	0	0	1/12 (8.3)	
		Black kite	6	1/6 (16.66)	1/6 (16.66)	0	0	0	1/6 (16.66)	
Turkey	2016	Eurasian sparrowhawk	3	0	0	3/3 (100)	0	0	0	Ciloglu et al., 2016
		Long-eared owl	1	0	0	1/1 (100)	0	0	0	
		Common buzzard	5	1/5 (20)	1/5 (20)	1/5 (20)	1/5 (20)	1/5 (20)	1/5 (20)	
		Long-legged buzzard	13	0	0	1/13 (7.69)	1/13 (7.69)	0	0	
		Tawny owl	1	0	0	1/1 (100)	1/1 (100)	0	0	
Iraq	2006	Serpent eagle	8	1/8 (12.5)	0	0	0	0	0	Jasim, 2006
		Marsh harrier	8	1/8 (12.5)	0	1/8 (12.5)	0	0	0	
		Kestrel	23	5/23 (21.73)	0	2/23 (8.69)	0	0	0	
		Lesser kestrel	16	4/16 (25)	0	1/16 (6.25)	0	0	0	
		Steppe buzzard	13	2/13 (15.38)	0	0	0	1/13 (7.69)	0	
		Long-legged buzzard	12	0	0	0	0	1/12 (8.33)	0	
		Black kite	8	0	0	0	0	1/8 (12.5)	0	
Saudi Arabia	2020	Saker falcon	55	1/55 (1.81)	1/55 (1.81)	0	0	0	0	Alfaleh et al., 2020
United Arab Emirates	2008	Saker falcon	432	24/432 (5.55)	0	24/432 (5.55)	0	0	0	Lierz et al., 2008
Germany	2021	Common buzzard	464	0	0	255/464 (54.95)	0	0	0	Wiegmann et al., 2021
		Red kite	46	0	0	15/46 (32.60)	0	0	0	
		Northern goshawk	18	0	0	4/18 (22.22)	0	0	0	
Austria and Bosnia-Herzegovina	2022	Eurasian goshawk	11	0	0	0	0	0	10/11 (90.9)	Harl et al., 2022
		Eurasian sparrowhawk	25	0	0	0	3/25 (12)	0	12/25 (48)	
		Common buzzard	75	0	1/75 (1.33)	0	2/75 (2.66)	0	72/75 (96)	
		Western marsh harrier	22	0	2/22 (9.09)	0	7/22 (31.81)	0	6/22 (27.27)	
		Hen harrier	4	0	0	0	0	0	1/4 (25)	
Greece	2025	Common buzzard	62	3/62 (4.83)	5/62 (8.06)	3/62 (4.83)	5/62 (8.06)	26/62 (41.9)	39/62 (62.90)	Markakis et al., 2025
		Eurasian sparrowhawk	26	3/26 (11.53)	9/26 (34.62)	3/26 (11.53)	9/26 (34.61)	20/26 (76.9)	26/26 (100)	
USA	2025	Different hawk species	543	6/543 (1.10)	6/543 (1.1)	167/543 (30.7)	167/543 (30.7)	50/543 (9.2)	50/543 (9.2)	Bell et al., 2025
		Different falcon species	38	6/38 (15.78)	6/38 (15.78)	2/38 (5.26)	2/38 (5.26)	3/38 (7.89)	3/38 (7.89)	
		Different owl species	14	3/14 (21.42)	3/14 (21.42)	3/14 (21.42)	3/14 (21.42)	6/14 (42.85)	6/14 (42.85)	
Brazil	2021	Different hawk species	25	0	0	0	0	0	8/25 (32)	Morel et al., 2021
		Different falcon species	130	0	0	0	0	0	26/130 (20)	
		Different owl species	51	0	1/51 (1.96)	0	0	0	50/51 (98.04)	
Egypt	2006	Egyptian kites	22	5/22 (22.72)	0	0	0	2/22 (9.09)	0	Ibrahim, 2006
Thailand	2021	Black kite	15	1/15 (6.66)	1/15 (6.66)	0	0	0	0	Pornpanom et al., 2021
		Shikra	16	1/16 (6.25)	1/16 (6.25)	0	0	0	0	

The chewing louse *L. maximum* was detected in 8/28 (28.57%) of the common buzzards (*Buteo buteo*). Larval *Argas* specimens morphologically compatible with *A. persicus* were detected in 2/11 (18 L. maximum chewing lice were found in 8 of 28 (28.57%) common buzzards (Buteo buteo), and larval Argas specimens morphologically compatible with *A. persicus* were detected in 2 of 11 (18.18%) golden eagles (*Aquila chrysaetos*). A few adult *A. persicus* ticks were also observed in the holding area. *L. maximum* has been reported in 32 raptor species across 18 genera (Clay, 1976), with the first Iranian case in a golden eagle (Rak et al., 1975). Additional reports include golden eagles in Shahrekord Province (Azizi et al., 2013), common buzzards in Turkey (Dik et al., 2022), and Spain (Talabante

et al., 2024). *A. persicus*, the fowl tick, is a soft tick reported in various regions of Iran (Beik-Mohammadi et al., 2025; Mazlum, 1971; Telmadarraiy et al., 2022), and its identification has been confirmed by molecular studies (Yavari et al., 2019). Other *Argas* species, such as *A. giganteus*, have been found in raptors like ospreys (Yabsley et al., 2025). Due to challenges in distinguishing larval Argas species morphologically, the specimens found on golden eagles are reported as morphologically compatible with *A. persicus*; molecular confirmation is recommended for precise identification. In this study, *Haemoproteus* spp. and *Leucocytozoon* spp. were detected by microscopy, *L. maximum* was found in common buzzards, and larval *Argas* specimens compatible with *A. persicus* were found in golden eagles. These results are specific to raptors admitted to the Mashhad rehabilitation center and may not represent the broader raptor population. Larger molecular studies are recommended to better assess haemoparasite frequency and confirm Argas species identification.

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Use of Artificial Intelligence

The authors certify that artificial intelligence (AI)-based methods were not used in the writing, analysis, interpretation, or preparation of this publication.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: G.R.R.; Methodology: D.J, G.R.R., A.M.; Investigation: D.J.; Writing - original draft preparation: D.J, G.R.R; Writing - review and editing: G.R.R.; Funding acquisition: G.R.R, A.M.; Supervision: G.R.R., A.M

Data Availability Statement

Information on each raptor was gathered in partnership with the Environmental Department of Khorasan Razavi Province, and access to this data requires approval from that department.

Ethical Considerations

All procedures involving raptors were approved by the Animal Ethics Committee of Ferdowsi University of Mashhad, Mashhad, Iran (approval No. IR.UM.REC.1402.140).

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