Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

Exo-erythrocytic stages of *Haemoproteus* sp. in common buzzard (*Buteo buteo*): A histopathological and molecular study

Hamidreza Shokrani [a,*], Hassan Norouzian [b], Omid Dezfoulian [a]

[a] Department of Pathobiology, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran
[b] Department of Clinical Sciences, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran

A B S T R A C T

Haemosporidian parasites are responsible for anemia, acute tissue degeneration, and depopulation in wild birds. This study aimed to investigate the prevalence of haemosporidians and also morphologic and molecular evaluation of tissue stages of Haemoproteus sp. in common buzzards (*Buteo buteo*). Eleven free-living common buzzards were referred to the Avian Clinic of Veterinary School of Lorestan University with lethargy, weight loss, and ataxia. Gametocytes of Leucocytozoon buteonis were found in blood smears of six (54.5 %) birds, while one had simultaneous infection with blood stages of Haemoproteus and Leucocytozoon. During histopathological examinations, exo-erythrocytic stages of the genus Haemoproteus were seen in the lung and kidney of a dead bird. This study is the first report of exo-erythrocytic infection of Haemoproteus in common buzzards. Molecular assays confirmed the infection of Haemoproteus sp. (lineage BUTBUT15) in tissue samples. Phylogenetic analysis using cytochrome *b* gene suggested that BUTBUT15 was more closely related to the lineages isolated from the family Falconidae in contrast to the Accipitridae.

1. Introduction

Haemosporidians (Phylum: Apicomplexa; Order: Haemosporida) are obligate intracellular protozoan parasites which infect a variety of reptilian, avian, and mammalian hosts (Borner et al., 2016). Little information is available about tissue development, morphological diversity, and the possibility of tissue destruction of avian haemosporidians. On the other hand, the life cycle of hemoproteids (Haemosporida: Haemoproteidae), particularly exo-erythrocytic stages, has been less described than other haemosporidians (Valkiunas and Iezhova, 2017).

Diagnoses of haemosporidian infections are made primarily based on the presence of micro- or macrogametocytes in blood smears. However, in low parasitemia, it is extremely difficult to detect the blood stages of Haemoproteus spp. by microscopy (Valkiunas, 2005). Previous studies indicate that the erythrocytic stages of haemosporidians have been more recognized as compared to exo-erythrocytic stages, especially in raptors (Munoz et al., 1999; Krone et al., 2001; Svobodová et al., 2015). Due to similarities between the tissue stages of haemosporidians, application of molecular methods in parallel with histopathology is inevitable, especially in mixed infections with two or more parasites (Cardona et al., 2002; Ferrell et al., 2007; Donovan et al., 2008; Olias et al., 2011; Pacheco et al., 2011; Cannell et al., 2013; Palinauskas et al., 2013; Valkiunas and Iezhova, 2017).

The risk and genetic diversity of haemosporidian infections in different wild migratory birds are probably greater than those of other bird species. These birds are principally susceptible to be infected with blood parasites. They can play significant roles in the ecology and circulation of some haemosporidians (Hubalek, 2004; Valkiunas, 2005; Nourani et al., 2020). Common buzzard (*Buteo buteo*) is a protected bird species that belongs to the family Accipitridae (Order: Accipitriformes) migrates through Iran during spring and fall (Mansoori, 2013). No study has been conducted to describe the prevalence of haemosporidians in Iranian birds of prey. The present study aimed to investigate the prevalence of haemosporidians in free-living common buzzards from western Iran, and also to evaluate the tissue stages of *Haemoproteus* sp. on the basis of histopathological and phylogenetic findings.

2. Materials and methods

2.1. Study area and blood sampling

Eleven free-living adult common buzzards were referred to the Avian Clinic of Veterinary School of Lorestan University between February 2017 and April 2020. All birds were collected in Lorestan province (33°48′N, 48°35′E), West of Iran. Lethargy, weight loss associated with anorexia and poor appetite, ruffled feathers, and incoordination were observed in clinical examinations of all birds. According to the statements, these birds had no history of alimentary, respiratory, or other pathognomonic signs.

Two smears were prepared with blood collected from the brachial vein of each bird. Smears were air-dried, fixed in 100 % methanol, and stained with Giemsa as described by Valkiunas (2005). Each sample was microscopically checked for at least 30 min at × 400 magnification. Positive samples were also examined at × 1000 magnification. The intensity of infection was estimated as a percentage by counting the number of gametocyte host cells per 10,000 erythrocytes. The species of the genus Leucocytozoon was identified using the taxonomic keys following Valkiunas (2005) and Valkiunas et al. (2010).

2.2. Necropsy and tissue sampling

In March 2017, a male common buzzard with a gunshot wound on his wing died shortly without any chance of treatment. The bird had co-infection with Leucocytozoon and Haemoproteus gametocytes. A routine necropsy was performed and internal organs including the brain, liver, lung, kidney, spleen, heart, and intestinal tract were removed. Subsequently, 1 g of each tissue sample was transferred to a 1.5 ml sterile tube and stored at −20 °C until DNA extraction. The rest of tissues were fixed in neutral buffered formalin 10 % and embedded in paraffin wax. The 4–5 μm thick sections were stained with hematoxylin and eosin (Valkiunas, 2005). The sizes of meronts and merozoites found in this study were expressed in micrometers (μm) as mean ± standard deviation (SD) followed by the range in parentheses. The number of meronts was counted in all available high-power fields (HPFs; × 400 magnification) and the result was shown as average number per 10 HPFs.

2.3. DNA extraction, PCR, and sequencing

Tissue samples were thoroughly powdered under liquid nitrogen. DNA extraction was performed on 25 mg of each tissue sample using the YTA Genomic DNA Extraction Mini Kit (Yekta Tajhiz Azma, Iran) following the supplier's instructions. PCR reaction was performed as previously described using specific primers HAEMF (5′ATGG TGCTTTCGATATATGCATG3′) and HAEMR2 (5′GCATTATCT GGATGTGATAATGGT3′) to amplify a 478bp fragment of the conserved regions of the *cytb* gene (Bensch et al., 2000).

Samples showing positive amplifications were selected for sequencing. The PCR products were purified and sequenced with both forward and reverse primers by Sequetech (Mountain View, CA). The obtained sequence was blasted in MalAvi database (Bensch et al., 2009). Pairwise sequence alignments were performed by the ClustalW (MEGA X program, version 10.2.5) between the obtained sequence and the other sequences available on MalAvi. A phylogenetic tree was constructed using MEGA X program with the maximum-likelihood method (Tamura et al., 2011).

3. Results

3.1. Microscopic and macroscopic inspection

Microscopic observation of blood smears revealed two morphologically distinguishable genera of haemosporidians (Leucocytozoon and Haemoproteus). Out of the total of 11 examined common buzzards, 54.5

% were infected with blood stages of Leucocytozoon. One of these six birds was simultaneously infected with erythrocytic stages of Haemoproteus. All infected birds were referred to the clinic during spring and summer seasons. The intensity of parasitemias in infected birds were light (<0.1 %).

Features of the Leucocytozoon gametocytes in different individual hosts were morphometrically similar. Gametocytes were exclusively observed in fusiform host cells. The nuclei of host cells were enlarged less than 1/3 of the circumference of gametocytes. Few gametocytes in which the host cell nucleus was completely enclosed by the parasite were observed (Fig. 1).

According to the morphological characteristics, the gametocytes could be considered as a species from the L. toddi group (Greiner and Kocan, 1977; Valkiunas, 2005). Based on pooled morphometric data, especially the length of the cytoplasmic processes and other features that depend on this character (Valkiunas et al., 2010), the species of Leucocytozoon was identified as L. buteonis. Morphometric data for macrogametocytes and host cells are given in Table 1. Because of limited sample size of non-deformed microgametocytes, this sexual form was not used for morphometric analysis.

The lineage of Haemoproteus sp. was not identified morphologically due to the absence of grown gametocytes. Just a few young gametocytes were detected in blood smears (Fig. 1).

The bird with co-infection died during the inspection because of a gunshot wound. At necropsy, both left lung and kidney showed hypostatic congestion, while the opposite organs were pale. Hepatomegaly and splenomegaly were striking in gross observation. The liver was friable and pale, while the spleen had white nodular hyperplasia (Fig. 2). No remarkable changes were seen in other organs.

In histopathological examination, most blood vessels were engorged with erythrocytes, also mild to moderate edema was presented throughout the lung. Some thin-walled meronts (3 per 10 HPFs) with morphological characteristics of Haemoproteus were found in the lung. Some of these pulmonary meronts were surrounded by small numbers of mononuclear cells. The morphology of meronts was variable from round to oval or branching and even amorphous. The mean sizes were 45.9 ± 19.2 (15.2–81.6) μm in length and 28.6 ± 10.6 (9.3–47.5) μm in width (n = 25). Host cell nucleus was not visible inside the meronts. Basophilic round to oval merozoites with a mean diameter of 1.2 ± 0.1 (1–1.6) μm and mean number of 89 ± 31.1 (47–155) were seen in each mature meront (n = 15; Fig. 3).

Moreover, a developing megalomeront with a diameter of 225 μm was detected in the kidney. The structure was enclosed with a 5 μm-thick hyalinized fibrous capsule-like wall, which contained highly eosinophilic irregularly-shaped cytomeres. Each cytomer contained numerous uninuclear merozoites. Host cell nucleus was not detectable inside or close to megalomeront. Inflammatory reaction around the megalomeront was low (Fig. 4).

Brain, spleen, liver, and cardiac muscles, were those evaluated organs which did not show tissue stages.

3.2. Phylogenetic analysis

A PCR-based study was used for the identification of exo-erythrocytic stages of *Haemoproteus* sp. in tissue samples. The kidney and lung tissues indicate the expected PCR-product with 478 nucleotides in length. The sequence determined in this study is available in MalAvi (lineage BUTBUT15) and GenBank (accession no. MG428417). In BLAST search, the nucleotide sequence of the *cytb* gene of the studied *Haemoproteus* sp. (BUTBUT15) was 97–98 % identical to lineages recorded in Lesser kestrel (*Falco naumanni*; lineage LK02) and other *Falco* spp. (lineages LK03 and LK04). The degree of similarity between the studied *Haemoproteus* sp. and BUBIBI01, the lineage with 12 avian host species including *Buteo buteo*, was also high (98 %). The studied *Haemoproteus* sp. (BUTBUT15) was 98 % and 97 % identical to *H. brachiatus* (lineage LK03) and *H. tinnuculi* (lineage FALSUB01) obtained from *Falco*
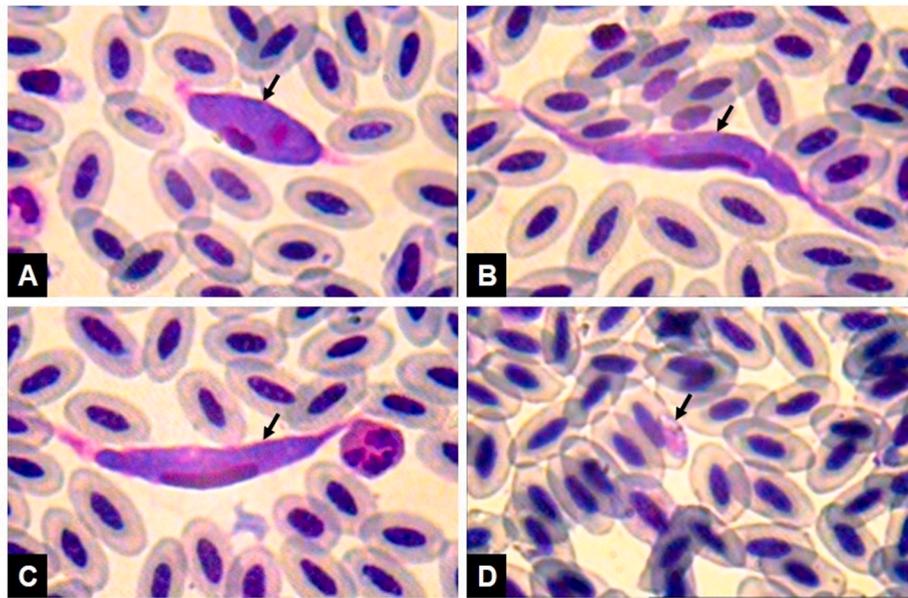
Fig. 1. Gametocytes of haemosporidians (arrows) from the blood of the common buzzards. **A-C** mature macrogametocytes of ***L. buteonis*** in fusiform host cells; **A** host cell nucleus is not distorted but displaced laterally; **B&C** host cell nucleus is lateral and flattened; **D** young gametocyte of *Haemoproteus*. Giemsa stained thin blood films. *Scale bar* = 10 μm.

Table 1

Morphometry of macrogametocytes and host cells of *L. buteonis*, found in six free-living common buzzards.

Feature	Measurements (μm)[a]
Macrogametocytes	*n* = 26
Length	15.1–26.0 (20.8 ± 3.0)
Width	4.0–10.2 (7.1 ± 1.7)
Parasite nucleus	
Length	3.2–7.0 (5.1 ± 0.9)
Width	2.8–4.8 (3.2 ± 0.5)
Host-cell nucleus	
Length	6.9–14.9 (11.0 ± 2.3)
Width	1.6–4.0 (2.5 ± 0.6)
Cytoplasmic processes[b]	
Length	9.5–21.7 (15.5 ± 3.7)
Width	1.8–6.4 (3.8 ± 1.0)
Host-cell parasite complex	
Length	30.1–61.8 (46.0 ± 9.1)
Width	5.6–13.9 (9.6 ± 2.3)

[a] Minimum and maximum values are provided, followed in parentheses by the arithmetic mean and standard deviation.

[b] Cytoplasm of fusiform host cell which forms processes at the ends of gametocyte.

naumanni and *Falco subbuteo*, respectively. In the phylogenetic tree, the lineage BUTBUT04 isolated exclusively from *Buteo buteo* was placed in a different clade and separated from our sequence. The percentage of similarity between BUTBUT15 and BUTBUT04 was 96 % (Fig. 5).

4. Discussion

Leucocytozoids have been recorded from a large number of bird species around the world (Greiner and Kocan, 1977). Diurnal raptors from Accipitridae are highly parasitized by leucocytozoids (Walther et al., 2016). Epidemiological studies have indicated that Leucocytozoon infection in birds from Accipitridae is ten times more common than Falconidae (Valkiunas, 2005).

Out of the 455 common buzzard nestlings which were examined in Germany, 40 % were infected with Leucocytozoon (Chakarov et al., 2008). The prevalence rate of leucocytozoids in raptors from different regions is more related to the avian species, climatic conditions, distribution of insect vectors, and also the methods applied for diagnosis (Walther et al., 2016; Nourani et al., 2020). There is a tendency for a higher prevalence of the L. toddi group in the direction from the tropical latitudes to the middle latitudes of the Northern Hemisphere (Valkiunas, 2005). Our microscopic results indicate a high prevalence of L. buteonis (54.5 %) in free-living common buzzards. The high prevalence of L. buteonis in apparently healthy adult birds suggests that this is a harmless endemic haemoparasite of common buzzards in west of Iran.

Some avian Haemoproteus spp. create acute illness and intensive mortality through exo-erythrocytic meronts (Valkiunas and Iezhova, 2017). Evidence suggests that the exo-erythrocytic stages might be different in their manifestations in the avian hosts, which could probably be dependent on various factors, including host diversity, new emergences of the parasite, and infection of birds with unspecified strains. Olias et al. (2011) reported a parakeet infected with H. minutus that produced deadly megalomeronts while parasitemia was absent. In this regard, it has been propounded that many haemosporidian strains have different developmental stages in the aberrant hosts. Tissue stages can develop in these hosts, but these structures will be arrested where the erythrocytic stages do not appear (Valkiunas and Iezhova, 2017).

Prior to the availability of molecular and genetic phylogenies, there was little information about the exo-erythrocytic stages of hemoproteids, and the identification was mainly based on morphological characteristics. If genetic lineages of haemosporidian parasites are linked with morphospecies, a remarkable volume of information would be available for phylogenetic analysis of these blood parasites (Valkiunas and Iezhova, 2017). In the present study, exo-erythrocytic stages of the genus Haemoproteus were identified in the histological sections. The cytb gene obtained from tissue samples confirmed the *Haemoproteus* sp. infection. The studied sequence belongs to an undescribed lineage (BUTBUT15). It was placed in a different clade, separate from other sequences recorded in *Buteo buteo*, closer to lineages isolated from *Falco naumanni* and some other falcon species. From a phylogenetic standpoint, the species of the studied *Haemoproteus* sp. is likely to be different from other reports from *Buteo buteo* and might be a new species. However, it was linked with two known morphospecies (*H. braciatus* and *H. tinnoculli*), according to the 5 % genetic distance criterion proposed by Hellgren et al. (2007).
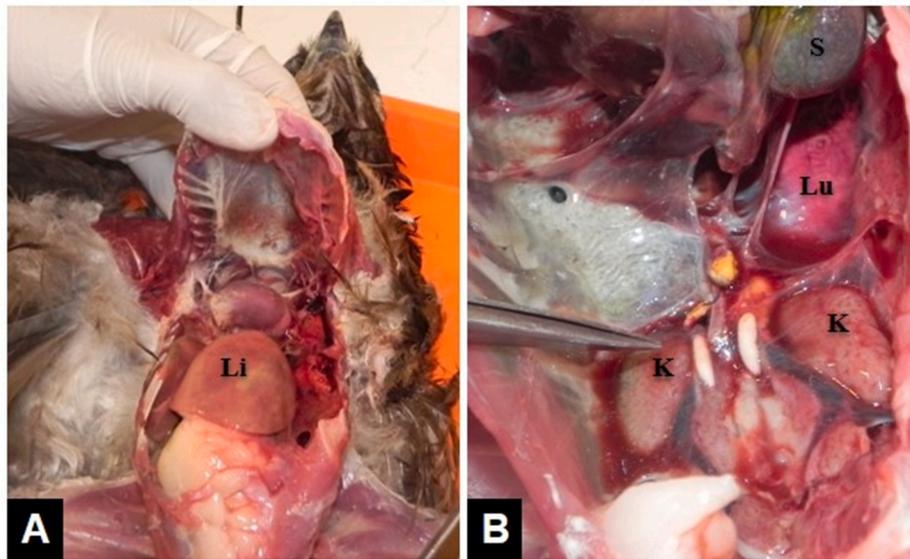
Fig. 2. Macroscopic examination. **A** pale enlarged liver (Li); **B** hyperplastic nodules on the surface of the large spleen (S), inflated kidneys (K), and hypostatic congestion of left lung (Lu).
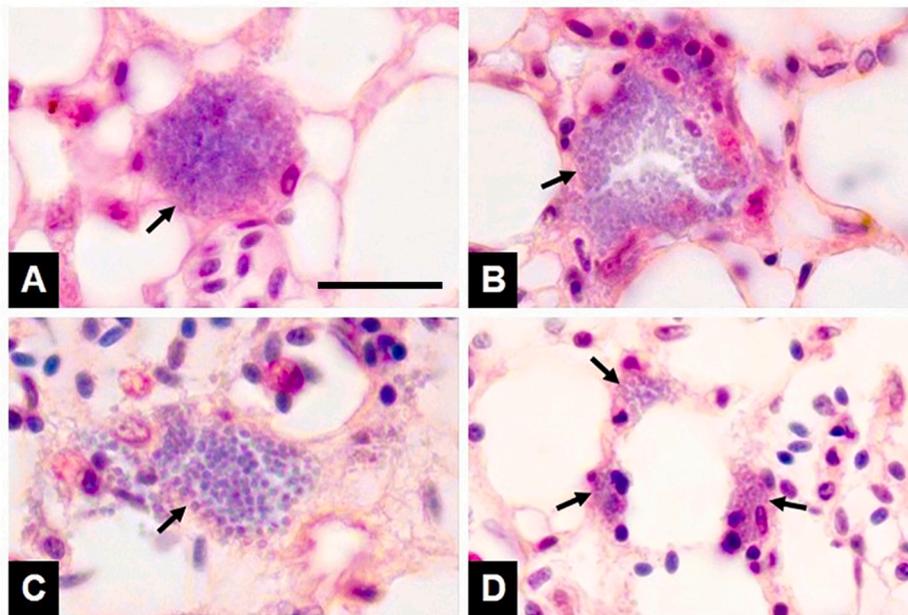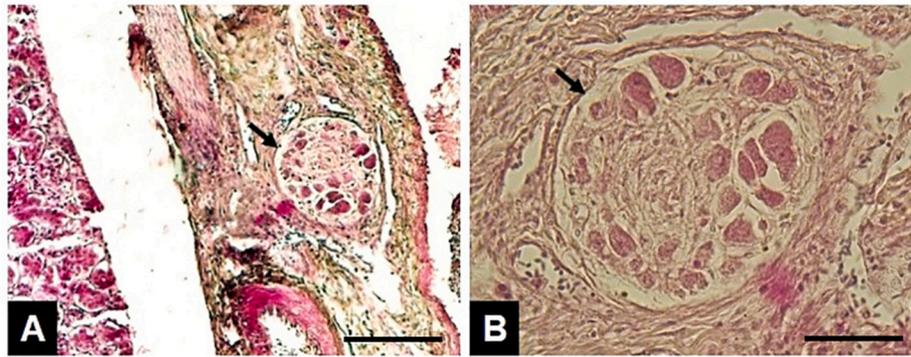

Fig. 3. Meronts of *Haemoproteus* sp. (linage BUTBUT15) from the lung of a common buzzard. **A-D** different shapes of pulmonary meronts (arrows). Each meront is surrounded by a thin wall and contained numerous basophilic round to oval merozoites. Note that the host cell nucleus is not visible in meronts. *Scale bar* = 40 μm.

Some *Haemoproteus* spp. lineages have reportedly a low degree of host specificity (Szymanski and Lovette, 2005; Nourani et al., 2018). The lineage BUBIBI01 has been found in different raptors, including buzzards (*Buteo buteo* and *Butastur indicus*), owls (*Bubo bubo, Athene noctua,* and *Asio flammeus*), falcons (*Falco* spp.), Eurasian sparrowhawk (*Accipiter nisus*), as well as Cattle egret (*Bubulcus ibis*).

There is evidence that shows the successful transmission of the hemoproteids between the birds (Nourani et al., 2018). Our phylogenetic analysis shows a close relationship between the *Haemoproteus* lineages recorded in birds of prey, in which isolates from *Buteo buteo* are placed adjacent to isolates from other raptors. Previous studies have confirmed the ambiguity of phylogenetic analysis of raptor hemoproteids. Ortego et al. (2007), in the study of *Haemoproteus* spp. in *F. naumanni*, confirmed three morphologically described species as well

as other undescribed lineages, identified by unique *cytb* sequences. All the recovered sequence-based lineages were not specific to Falconiformes. The study of Ishak et al. (2008) revealed a lack of phylogenetic cohesion among raptor blood parasites. In addition, Outlaw and Ricklefs (2009) mentioned the overall lack of phylogenetic relatedness among raptor haemosporidians.

Iran is situated at the crossroads of several major bird migration routes and may be influenced to have a high risk of exposure to new parasites (Nourani et al., 2018). *Falco* spp. (eg, *naumanni* and *subbuteo*) are common summer visitors, and the Eurasian kestrel (*Falco tinnunculus*) is a permanent inhabitant of the west of Iran (Mansoori, 2013). *Falco naumanni* is a trans-Saharan migrant and is likely to be infected by a wide array of avian blood parasites from Asia and Africa (Waldenström et al., 2002). Migratory falcons are likely to act as vehicles for

Fig. 4. Megalomeront of *Haemoproteus* sp. (linage BUTBUT15) from the kidney of a common buzzard. **A&B** same megalomeront of different magnifications (arrows). The structure is covered with a semi-thick capsule-like wall and contained highly eosinophilic irregularly-shaped cytomeres which have merozoites. Note that the host cell nucleus is not visible inside or close to megalomeront. Scale b*ars* = 200 μm (**A**); 80 μm (**B**).
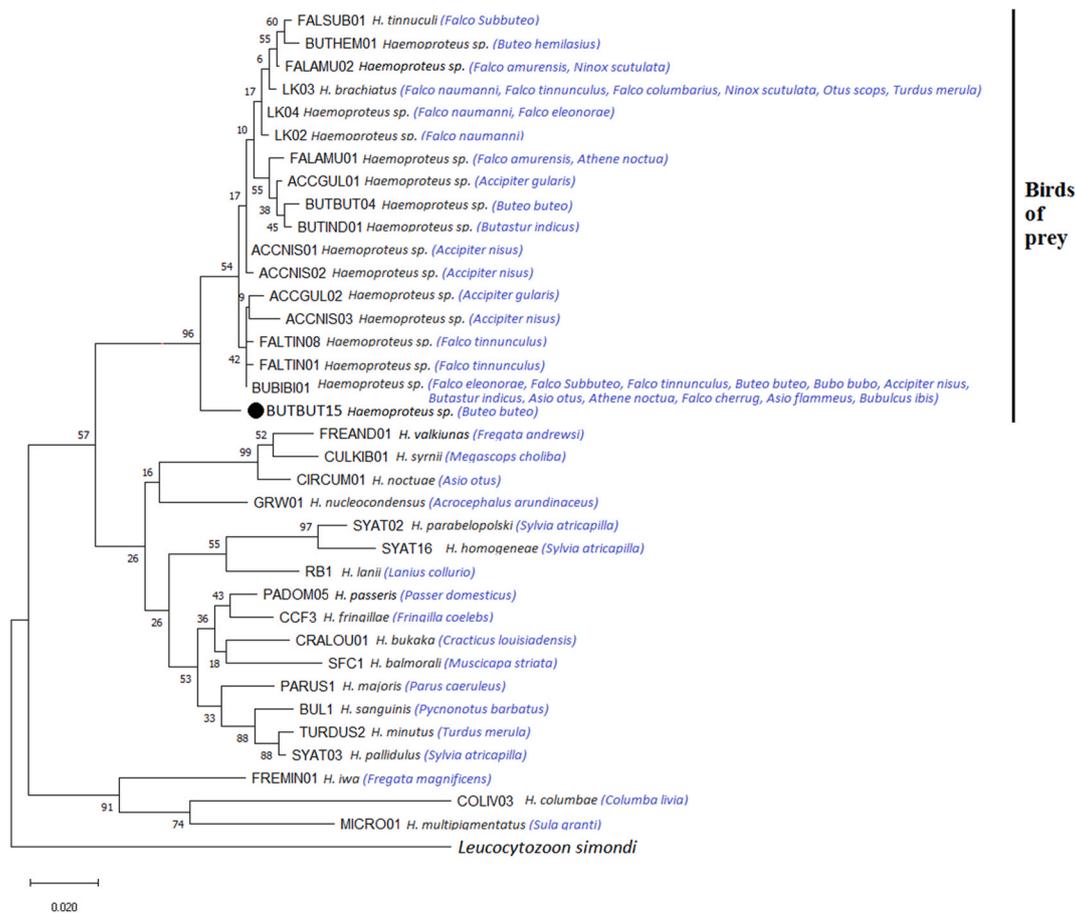
Fig. 5. Maximum-likelihood tree analysis based on partial *cytb* gene sequences (433 bp) of *Haemoproteus* from the studied sequence (linage BUTBUT15) and other sequences obtained from MalAvi database. Numbers on the branches indicate the percent of replicates that reproduced the topology for each clade. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Black circle indicates sequence obtained from the study.

introducing new species of *Haemoproteus* to the Iranian population of *Buteo buteo*.

5. Conclusion

The present study provided new achievements based on molecular and histopathological findings of Haemoproteus sp. in *Buteo buteo*. Close phylogenetic relationship between the studied Haemoproteus sp. and lineages isolated from Falco naumanni, other members of Falco genus and Cattle egret, which is not a bird of prey, suggest that linage BUTBUT15 might be a Haemoproteus sp. with a wide range of avian hosts. This study reports the first investigation on exo-erythrocytic stages of Haemoproteus sp. in common buzzards. Moreover, this is the first histopathological study of tissue stages of haemosporidians in Iran. Comprehensive and inclusive research is still required to clarify the undefined structural characteristics and pathogenesis of haemosporidian parasites.

Declaration of competing interest

Acknowledgements

References

Bensch, S., Stjernman, M., Hasselquist, D., Stman, O., Hansson, B., Westerdahl, H., Pinheiro, R.T., 2000. Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. Proc. Biol. Sci. 267, 1583–1589.

Bensch, S., Hellgren, O., Perez-Tris, J., 2009. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Mol. Ecol. Resour. 9, 1353–1358.

Borner, J., Pick, C., Thiede, J., Kolawole, O.M., Kingsley, M.T., Schulze, J., Cottontail, V. M., Wellinghausen, N., Schmidt-Chanasit, J., Bruchhaus, I., Burmester, T., 2016. Phylogeny of haemosporidians blood parasites revealed by a multi-gene approach. Mol. Phylogenet. Evol. 94, 221–231.

Cannell, B.L., Krasnec, K.V., Campbell, K., Jones, H.I., Miller, R.D., Stephens, N., 2013. The pathology and pathogenicity of a novel *Haemoproteus* spp. infection in wild Little Penguins (*Eudyptula minor*). Vet. Parasitol. 197, 74–84.

Cardona, C.J., Ihejirika, A., Mcclellan, L., 2002. *Haemoproteus lophortyx* infection in bobwhite Quail. Avian Dis. 46, 249–255.

Chakarov, N., Boerner, M., Kruger, O., 2008. Fitness in common buzzards at the cross-point of opposite melanin–parasite interactions. Funct. Ecol. 22, 1062–1069.

Donovan, T.A., Schrenzel, M., Tucker, T.A., Pessier, A.P., Stalis, I.H., 2008. Hepatic hemorrhage, hemocoelom, and sudden death due to *Haemoproteus* infection in passerine birds: eleven cases. J. Vet. Diagn. Invest. 20, 304–313.

Ferrell, S.T., Snowden, K., Marlar, A.B., Garner, M., Lung, N.P., 2007. Fatal hemoprotozoal infections in multiple avian species in a zoological park. J. Zoo Wildl. Med. 38, 309–316.

Greiner, E.C., Kocan, A., 1977. *Leucocytozoon* (Haemospordia: Leucocytozoidae) of the Falconiformes. Can. J. Zool. 55, 761–770.

Hellgren, O., Krizanauskiene, A., Valkĭunas, G., Bensch, S., 2007. Diversity and phylogeny of mitochondrial cytochrome B lineages from six morphospecies of avian *Haemoproteus* (Haemosporida: Haemoproteidae). J. Parasitol. 93, 889–896.

Hubalek, Z., 2004. An annotated checklist of pathogenic microorganisms associated with migratory birds. J. Wildl. Dis. 40, 639–659.

Ishak, H.D., Dumbacherm, J.P., Anderson, N.L., Keane, J.J., Valkiunas, G., Haig, S.M., Tell, L.A., Sehgal, R.N., 2008. Blood parasites in owls with conservation implications for the spotted owl (*Strix occidentalis*). PloS One 28 (3), e2304.

Krone, O., Priemer, J., Streich, J., Sommer, P., Lannggemacht, T., Lessow, O., 2001. Haemosporida of birds of prey and owls from Germany. Acta Protozool. 40, 281–289.

Mansoori, J., 2013. A Guide to the Birds of Iran, third ed. Farzaneh Publishing, Tehran ([In Persian]).

Muñoz, E., Ferrer, D., Molina, R., Adlard, R.D., 1999. Prevalence of haematozoa in birds of prey in Catalonia, north-east Spain. Vet. Rec. 144, 632–636.

Nourani, L., Aliabadian, M., Mirshamsi, O., Djadid, N.D., 2018. Molecular detection and genetic diversity of avian haemosporidian parasites in Iran. PloS One 13 (11), e0206638.

Nourani, L., Dinparast Djadid, N., Rabiee, K., Shirzoor Mezerji, M., Shakiba, M., Bakhshi, H., Shokrollahi, B., Khaltabadi Farahani, R., 2020. Detection of haemosporidian parasites in wild and domestic birds in northern and central provinces of Iran: Introduction of new lineages and hosts. Int. J. Parasitol. Parasites Wildl. 13, 203–212.

Olias, P., Wegelin, M., Zenker, W., Freter, S., Gruber, A.D., Klopfleisch, R., 2011. Avian malaria deaths in parrots, Europe. Emerg. Infect. Dis. 17, 950–952.

Ortego, J., Calabuig, G., Cordero, P.J., Aparicio, J.M., 2007. Genetic characterization of avian malaria (Protozoa) in the endangered lesser kestrel, *Falco naumanni*. Parasitol. Res. 101, 1153–1156.

Outlaw, D.C., Ricklefs, R.E., 2009. On the phylogenetic relationships of haemosporidian parasites from raptorial birds (Falconiformes and Strigiformes). J. Parasitol. 95, 1171–1176.

Pacheco, M.A., Escalante, A.A., Garner, M.M., Bradley, G.A., Aguilar, R.F., 2011. Haemosporidian infection in captive masked bobwhite quail (*Colinus virginianus ridgwayi*), an endangered subspecies of the northern bobwhite quail. Vet. Parasitol. 182, 113–120.

Palinauskas, V., Iezhova, T.A., Križanauskienė, A., Markovets, M.Y., Bensch, S., Valkiunas, G., 2013. Molecular characterization and distribution of *Haemoproteus minutus* (Haemosporida, Haemoproteidae): a pathogenic avian parasite. Parasitol. Int. 62, 358–363.

Szymanski, M.M., Lovette, I.J., 2005. High lineage diversity and host sharing of malarial parasites in a local avian assemblage. J. Parasitol. 91, 768–774.

Svobodová, M., Weidinger, K., Peške, L., Volf, P., Votýpka, J., Voříšek, P., 2015. Trypanosomes and haemosporidia in the buzzard (*Buteo buteo*) and sparrow hawk (*Accipiter nisus*): factors affecting the prevalence of parasites. Parasitol. Res. 114, 551–560.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Valkiunas, G., 2005. Avian Malaria Parasites and Other Haemosporidia. CRC Press, Boca Raton, Florida.

Valkiunas, G., Sehgal, R.N.M., Iezhova, T.A., Hull, A.C., 2010. Identification of *Leucocytozoon toddi* group (Haemosporida, Leucocytozoidae), with remarks on the species taxonomy of leucocytozoids. J. Parasitol. 96, 170–177.

Valkiunas, G., Iezhova, T., 2017. Exo-erythrocytic development of avian malaria and related haemosporidian parasites. Malar. J. 101, 1–24.

Waldenström, J., Bensch, S., Kiboi, S., Hasselquist, D., Ottosson, U., 2002. Cross-species infection of blood parasites between resident and migratory songbirds in Africa. Mol. Ecol. 11, 1545–1554.

Walther, E., Valkiunas, G., Wommack, E.A., Bowie, R.C.K., Iezhova, T.A., Sehgal, R.N.M., 2016. Description and molecular characterization of a new *Leucocytozoon* parasite (Haemosporida: Leucocytozoidae), *Leucocytozoon californicus* sp. nov., found in American kestrels (*Falco sparverius sparverius*). Parasitol. Res. 115 (5), 1853–1862.