Identification, frequency, phylogeny and risk factors of Haemosporida infection of birds of prey in Greece

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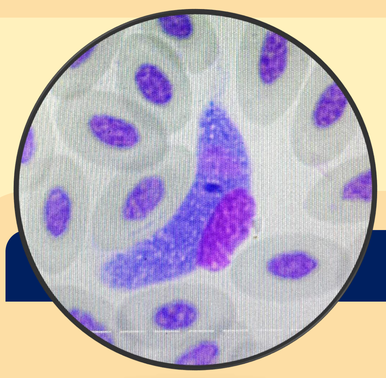
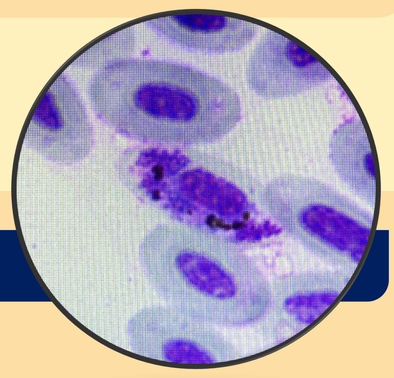
Introduction

The terms “birds of prey” or “raptors” include birds belonging to the orders Accipitiformes, Falconiformes and Strigiformes (Barbon et al. ,2021; Atkinson et al., 2008).

These fowl host many parasites. Important endoparasites of raptors are Haemosporidians, which use blood-sucking dipteran insects as vectors. Species belonging to the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* are the ones of most wildlife importance (Atkinson et al., 2008; Valkiūnas, 2005). Several cases of fatal haemosporidiosis in birds have been reported globally (Remple, 2004). In raptors data are lacking in Greece, a country which poses a crossroad to the migration path of birds.

The objective of this study was to determine, for the first time, the prevalence of haemosporidian infection of raptors in Greece in correlation with some risk factors.





Methodology

In total, samples from 119 birds belonging to 13 common European raptor species were analyzed in this study. These birds were admitted to “ANIMA”, the biggest wildlife rehabilitation centre in Greece.

Blood samples were collected and the morphological identification of parasites was performed after staining blood smears with Giemsa.

Moreover, total DNA was extracted following the Quick-DNA™ Miniprep Kit by Zymo Research. Samples were then screened for positive infections following a modification of the nested PCR protocol (Waldenström et al., 2004), developed by Perez-Rodriguez et al. (2013), which amplifies a fragment of the mitochondrial cytochrome b gene of all three genera. It involves a first pre-amplification PCR step using the primers Plas1F
(5’ -GAGAATTATGGAGTGGATGGTG-3’ ; Duval et al., 2007) and HaemNR3
(5’ - ATAGAAAGATAAGAAATACCATTC-3’ ; Hellgren et al., 2004), followed by a nested PCR step with the internal primers 3760F
(5’ - GAGTGGATGGTGTTTTAGAT-3’ ; Beadell et al., 2004) and HaemJR4 (5’ - GAAATACCATTCTGGAACAATATG-3’).

Thereafter, DNA fragments of all PCR samples were sequenced using Big Dye Terminator V3.1 Cycle Sequencing Kit and ABI PRISMTM 3100 capillary sequencing robot (Applied Biosystems, Foster City, CA, USA). Those that had mixed infections were subjected to a second nested PCR using initially the primers HaemNFI
(5’-CATATATTAAGAGAAITATGGAG-3’) and HaemNR3
(5’-ATAGAAAGATAAGAAATACCATTC-3’) to amplify parasite mtDNA from all three genera and then HaemF
(5’ – ATGGTGCTTTCGATATATGCATG-3’) and HaemR2
(5’-GCATTATCTGGATGTGATAATGGT-3’) (Bensch et al., 2000) for *Plasmodium* spp. and *Haemoproteus* spp. (Hellgren et al., 2004).

Results

Out of 119 birds that were examined, 80 were found positive by PCR for haemosporidians (67.22%).

Sequencing revealed that from these 80 samples, 35 were infected with various *Leucocytozoon* species (43.75%), 22 with one *Leucocytozoon* species (27.5%), 15 with *Leucocytozoon* spp. and *Plasmodium* spp. or *Haemoproteus* spp. (18.75%), 5 with *Haemoproteus* spp. (6.25%) and 3 with *Plasmodium* spp. (3.75%).

As regard phylogenetic analysis, for *Leucocytozoon* spp. several genetic lineages were isolated (ACNI1, ACNI04, CIRCYA01, MILANS04, MILVUS01, BUBT3), with the BUBT2 being the most frequent one. For *Plasmodium* spp. and *Haemoproteus* spp., the most common identified genetic lineage was TURDUS1. Also some new genetic lineages were revealed. Sex was assessed as a risk factor of infection with 84% female birds being infected opposed to 63% male birds.

Discussion/ Conclusion

This study demonstrated for the first time the high prevalence of Haemosporidians in birds of prey in Greece. The most prevalent infection was by *Leucocytozoon* spp., whilst most of the samples had mixed infections.

Wild animals contribute to the maintenance of parasites in nature as they are reservoirs for domestic animals. In addition, the migration of wild birds contributes to the dissemination of parasites from region to region or even between continents (Atkinson et al., 2008).

Haemosporidian-infected birds of prey took longer to rehabilitate and had higher mortality rates compared to those in which no haemosporidians were detected (Deem, 1999). In addition, it has been reported that avian haemoparasites may cause extinctions and significant declines in bird populations (Atkinson et al. , 2010; Dadam et al., 2019).

The aforementioned data suggest the great importance of studying these parasites.

These results underline the necessity of further studies regarding the prevalence, the diversity and the consequences of haemosporidiosis in raptors, a group of birds that is underrepresented in studies of haemosporidians.

References

- Barbon, A. & Kubiak, M. (2021). *Handbook of Exotic Pet Medicine.* Willey Blackwell, New Jersey, USA.
- Atkinson, C., Thomas, N. & Hunter, D. (2008). “*Parasitic diseases of wild birds”.* Ames, Iowa : Willey, Blackwell, USA.
- GediminasValkiūnas (2005). *Avian Malaria Parasites and Other Haemosporidia*. CRC Press, Florida.
- Remple J.D. (2004). “*Intracellular Hematozoa of Raptors: A Review and Update”*. J. of Avian Medicine and Surgery; 18(2):75-88
- Atkinson CT, Samuel MD. (2010). “*Avian malaria Plasmodium relictum in native Hawaiian forest birds: epizootiology and demographic impacts on àapapane Himatione sanguinea”.* J Avian Biol.;41:357–66.
- Deem S. (1999). *“Infectious and Parasitic Diseases of Raptors”.* Compendium on Continuing Education for the Practising Veterinarian. 21(4): 329-338.
- Dadam D, Robinson R.A., Clements A., Peach W.J, Bennett M., Rowcliffe J.M., et al. (2019). “*Avian malaria-mediated population decline of a widespread iconic bird species”.* R Soc Open Sci.;6:182197.
- Hellgren O., Waldenstrom J. Bensch S. (2004). “A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium and Haemoproteus from avian blood” J. Parasitol., 90(4), pp. 797–802
- Pérez-Rodríguez A., Javier de la Puente , Onrubia A., Javier Pérez-Tris. (2013*). “Molecular characterization of haemosporidian parasites from kites of the genus Milvus (Aves: Accipitridae)”.* International Jourrnal of Parasitology 43, 381-387