

First assessment of the prevalence of haemosporidian infection of raptors in Greece

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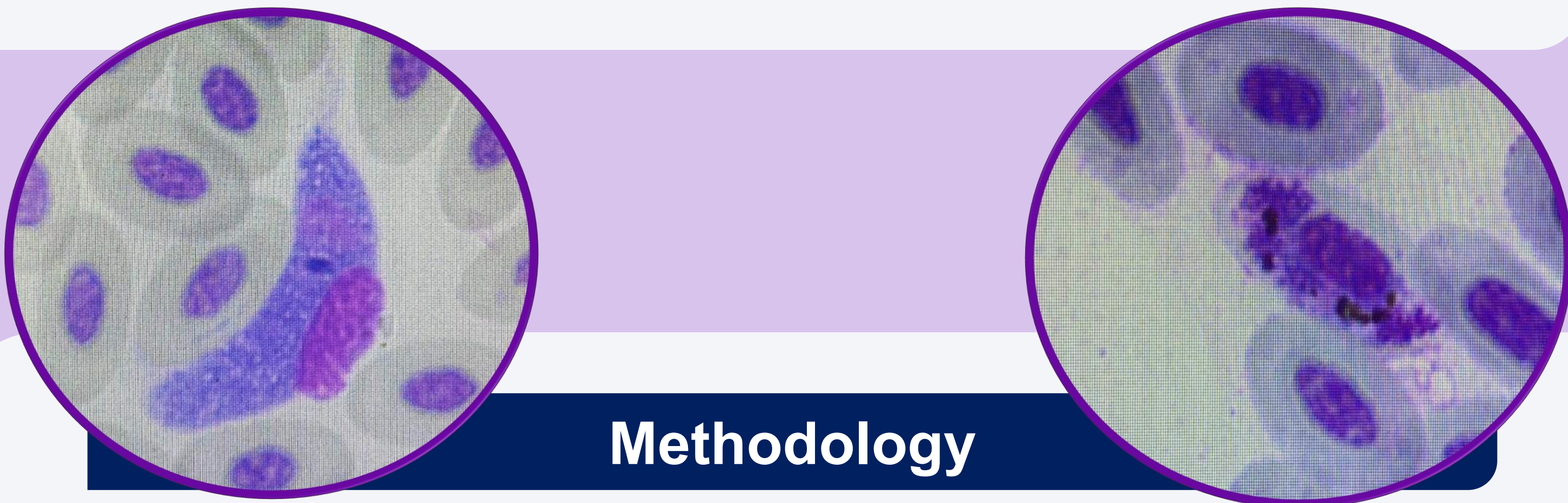


Introduction

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

These avian species harbour many parasites. Important endoparasites of raptors are Haemosporidians, that use blood-sucking dipteran insects as vectors. The most important species for wildlife belong to the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (Valkiūnas, 2005; Atkinson et al., 2008). Several cases of birds dying from haemosporidiosis have been reported globally (Remple, 2004). In raptors data are scarce in Greece, a country which poses a crossroad to the migration path of birds.

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



Methodology

In total, 119 birds belonging to 13 common European raptor species took part in this study. These birds were admitted to “ANIMA, Association for Wildlife Care and Protection” in Athens, Greece.

Blood samples were collected and blood smears were stained with Giemsa for the morphological identification of parasites.

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 Haemosporidian 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 the HaemJR4 (5' - GAAATACCATTCTGGAACAATATG-3').

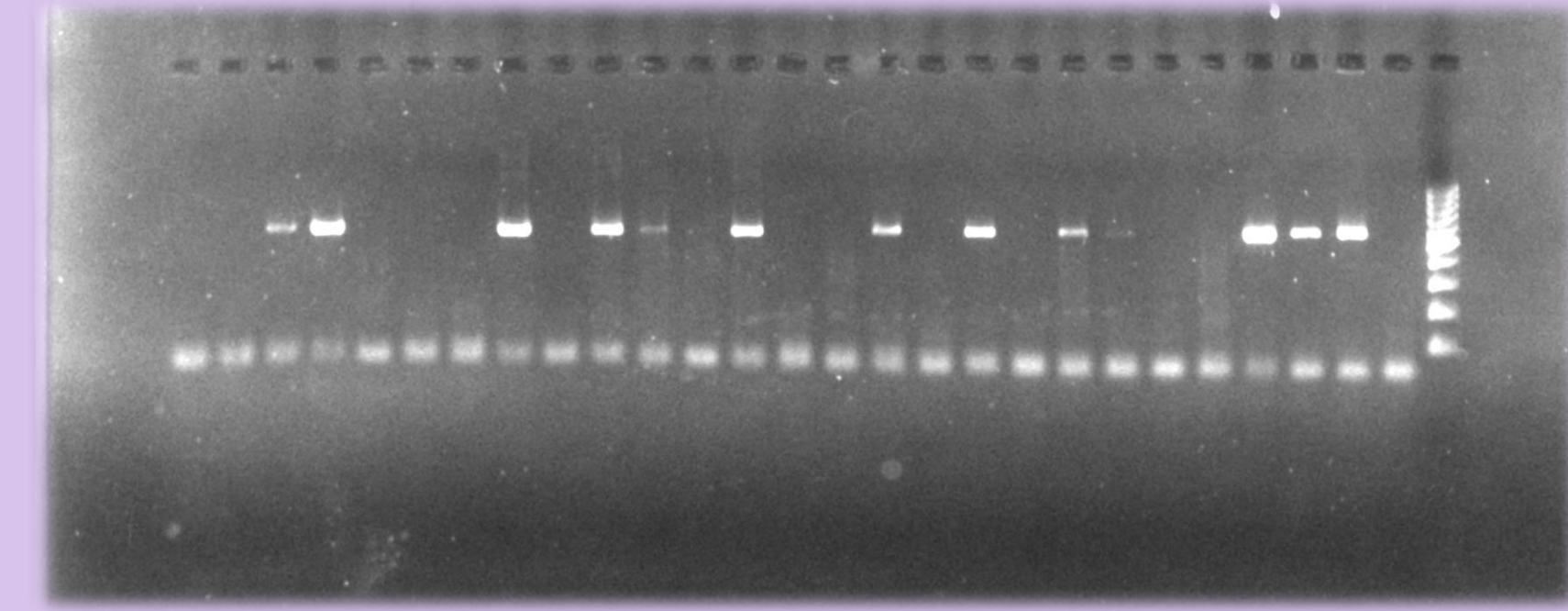
Thereafter, DNA fragments of all PCR samples were sequenced using Big Dye Terminator V3.1 Cycle Sequencing Kit and ABI PRISMTM 3100 capillary sequencing robot (Applied Biosystems, Foster City, CA, USA). Those that had mixed infections were subjected to a second nested PCR using initially the primers 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') primers (Bensch et al., 2000) for *Plasmodium* spp. and *Haemoproteus* spp. (Hellgren et al., 2004).

Results

The prevalence of haemosporidian infection was 67.22% by PCR (80 positive samples out of 119 examined). Sequencing revealed that the prevalence of *Leucocytozoon* spp mixed infections was 43.75% (35/80), whilst the 27.5% of the samples were infected with one *Leucocytozoon* species (22/80). The 18.75% of the samples were infected with *Leucocytozoon* spp. and *Plasmodium* spp. or *Haemoproteus* spp. (15/80), the 6.25% with *Haemoproteus* spp. (5/80) and the 3.75% with *Plasmodium* spp. (3/80).

As far as phylogenetic analysis is concerned, 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. Some new genetic lineages were revealed as well.

Sex was assessed as a potent risk factor of infection with 84% female birds being infected opposed to 63% male birds.



Discussion/ Conclusion

This study assessed for the first time the prevalence of Haemosporidian infections in birds of prey in Greece, which was interestingly high. *Leucocytozoon* spp. infections were the most prevalent ones, whilst most of the samples had mixed infections.

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

Haemosporidian - infected birds of prey presented longer rehabilitation periods and 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 significant declines in bird populations, even extinctions (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.

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