MICROSCOPIC DETECTION OF HEMOPARASITE INFECTION IN GREY PARROTS (*Psittacus erithacus*) IN PORTUGAL

DETEÇÃO MICROSCÓPICA DA INFEÇÃO POR HEMOPARASITAS EM PAPAGAIOS-CINZENTOS (*Psittacus erithacus*) EM PORTUGAL

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Resumo: *Espécies pertencentes aos géneros Haemoproteus, Plasmodium e Leucocytozoon são parasitas transmitidos por vetores que infetam células sanguíneas e vários órgãos dos seus hospedeiros. Estes três géneros de parasitas são geralmente relatados como sendo patogénicos e estudos recentes sugerem que algumas espécies podem por vezes ser letais, mais frequentemente do que anteriormente relatado. A prevalência da infeção está intimamente relacionada com a distribuição destes vetores. Estes hemoparasitas são amplamente descritos em países com temperaturas amenas, uma vez que os seus vetores necessitam de temperaturas quentes para a sua sobrevivência e reprodução. As alterações climáticas, nomeadamente um aumento da temperatura média, aumentam a probabilidade de propagação destes vetores e, por essa razão, a propagação de hemoparasitas. Uma vez que existe um desconhecimento da infeção por haemosporidia não só em Papagaios-cinzentos (Psittacus erithacus) mas também na família Psittacidae, o principal objetivo deste estudo foi estimar a prevalência da infeção por hemoparasitas em P. erithacus saudáveis em Portugal Continental. Foram colhidas um total de 70 amostras de sangue proveniente de aves assintomáticos mantidas em coleções privadas em várias regiões de Portugal Continental. A infeção por hemoparasitas foi avaliada por observação microscópica. A análise revelou a presença de hemoparasitas em 8 amostras, o que representa uma prevalência de 11,43% (8/70). Este trabalho representa uma importante contribuição epidemiológica para a fauna cativa de P. erithacus neste país e, tanto quanto é do nosso conhecimento, este é o primeiro estudo de vigilância da infeção por hemoparasitas nesta espécie em Portugal.*

Palavras-chave*: Hemoparasitas, Plasmodium, Haemoproteus, Leucocytozoon, Psittacus erithacus.*

Abstract: *Species belonging to the genera Haemoproteus, Plasmodium and Leucocytozoon are vector-borne parasites infecting blood cells and several organs of their hosts. These three parasite genera are commonly reported as being pathogenic and recent studies suggest that some species could sometimes be lethal, more frequently than previously reported. The prevalence of infection is closely related to the distribution of these vectors. These hemoparasites are widely described in countries with mild temperatures since their vectors need warm temperatures to survive and reproduce. Climate change, namely an increase in average temperature, increases the likelihood of the spread of these vectors and,*

for that reason, the spread of hemoparasites. Since there's a lack of knowledge of the infection by haemosporidia not only in Grey Parrots (Psittacus erithacus) but also in family Psittacidae, the main objective of this study was to estimate the prevalence of haemosporidia in healthy Psittacus erithacus in mainland Portugal. A total of 70 blood samples were taken from asymptomatic Psittacus erithacus kept in private bird collections in several regions of mainland Portugal. The presence of haemosporidia was assessed by microscopic observation. Microscopic analysis revealed the presence of haemosporidian pathogens in 8 samples, accounting for a prevalence of 11.43% (8/70). This work represents an important epidemiological contribution to the captive fauna of Psittacus erithacus in this country and, to the best of our knowledge, this is the first surveillance study of haemosporidia in this species in Portugal.

***Keywords**: Haemosporidia, Plasmodium, Haemoproteus, Leucocytozoon, Psittacus erithacus.*

1. INTRODUCTION

Haemoproteus spp., *Plasmodium* spp. and *Leucocytozoon* spp., are unicellular protists belonging to the phylum Apicomplexa, with over 6,000 species, order Haemosporida and families Haemoproteidae, Plasmodiidae and Leucocytozoidae, respectively (Valkiünas & Atkinson, 2020; Prasopsom & Salakij, 2020; Romero-Palmera, Valera & Silva-Sánchez, 2019; Morrison, 2009; Valkiünas, 2005). These parasites are transmitted by vectors, haematophagous diptera, and infect blood cells (Valkiünas & Atkinson, 2020; Verwey *et al.*, 2018; Chagas *et al.*, 2017; Hellgren *et al.*, 2004).

To date, these parasites have been detected in 106 countries on five continents and have 308 vector species and 1,489 vertebrate host species (Romero-Palmera, Valera & Silva-Sánchez, 2019). Particularly in Europe, almost half of the countries (23/50) have reported the detection of haemosporidians, representing a presence of infection in European countries of 46% (Romero-Palmera, Valera & Silva-Sánchez, 2019).

Haemoparasites, depending on the genus, also have different haematophagous dipterous vectors; *Plasmodium* is typically transmitted by mosquitoes (Culicidae); whereas *Haemoproteus* is transmitted by biting midges of the family Ceratopogonidae and louse flies of family Hippoboscidae; finally *Leucocytozoon* is transmitted by blackflies belonging to the family Simuliidae (Ibáñez-Bernal, Rivera-García, & Abella-Medrano, 2020; Ferreira, Santiago-Alarcon, & Braga, 2020; Romero-Palmera, Valera & Silva-Sánchez, 2019; Verwey *et al.*, 2018; Valkiünas & Iezhova, 2017; Valkiünas, 2005). Being a disease transmitted by dipteran vectors, haemoparasite infection is intrinsically related to the natural distribution of the respective vectors (Romero-Palmera,

Valera & Silva-Sánchez, 2019; Verwey *et al.*, 2018). Seasonality acquires a relevant role since these vectors require mild temperatures for their reproduction and survival (Verwey *et al.*, 2018). Fluctuations in temperature will result in fluctuations on the abundance of the vectors (Verwey *et al.*, 2018). If the temperature decreases the abundance of these vectors also decreases, if the temperature increases so does the abundance of the insects (Verwey *et al.*, 2018).

Several studies report haemoparasite infection in birds of the psittacidae order and even the development of fatal disease (Cocumelli *et al.*, 2021; García-del-Río *et al.*, 2021; Verwey *et al.*, 2018; Valkiünas & Iezhova, 2017; Chagas *et al.*, 2017; Adlard, Pierce & Lederer, 2004). Psittacines are one of the most endangered orders, many of them being classified as endangered by the International Union for the Conservation of Nature (IUCN); infection by these parasites can represent a great risk for psittacidae species (Cocumelli *et al.*, 2021; Ortiz-Catedral *et al.*, 2019; IUCN Red List www.iucnredlist.org). Particularly in the case of *Psittacus erithacus*, in Italy, one case of mortality has been reported in which the cause of death identified was the presence of *Haemoproteus* sp. infection (Tarello, 2005).

The detection of parasites can be performed by microscopic observation of blood smears (Verwey *et al.*, 2018; Hellgren *et al.*, 2004). Microscopy allows the identification of coinfections and the distinction of different morphological species (Valkiünas & Atkinson, 2020).

The maintenance of exotic birds as pets is growing and with it the commercial trade of these birds. This can lead to the introduction of different haemosporidian species. This represents not only a threat to other captive exotic species but also to endemic species. The aim of this study was to evaluate the presence of haemosporidians infection in captive healthy *P. erithacus* in mainland Portugal and identify lineages of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* in these birds.

2. MATERIAL AND METHODS

2.1. Sample collection

Blood samples were collected between November 2021 and October 2022 from healthy captive adult *P. erithacus* from mainland Portugal. A total of 70 birds were enrolled in this study. Adult birds of both sexes were included.

Blood samples were collected from the birds' right jugular vein and direct blood smears from each bird were performed.

All samples were collected by experienced avian veterinarians in the context of the clinical evaluation of birds.

2.2. Microscopic analysis

All 70 blood smears were air-dried, fixed in 100% methanol and stained subsequently with Giemsa. Each blood smear was scan for at least 10 minutes using an Abaxis 3000-LED series microscope with Accu-Scope Plan 40x/0.65 objectives equipped with a Excelis HDS HD CAMERA & MONITOR SYSTEM for detection of haemosporidia. Parasites were identified according to Valkiünas (2005).

3. RESULTS

Blood smear examinations detected 8 out of 70 individuals positive for haemosporidian infection, accounting for a prevalence of infection of 11.43%. The blood smear examination allowed us to detect different degrees of maturity of *Haemoproteus* sp., immature and mature gametocytes were observed in red blood cells (Figure 1).

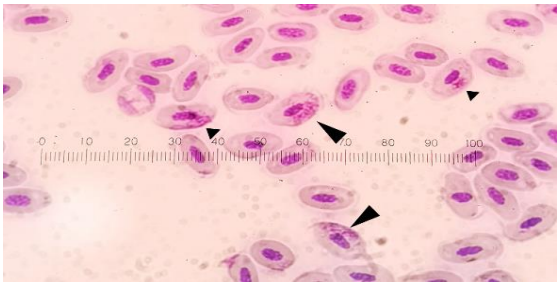

Figure 1. Mature microgametocytes (short arrow heads) and macrogametocytes (arrow heads) of *Haemoproteus* sp. in red blood cells.

In some birds only a few immature gametocytes/trophozoites were observed in blood smears. For that reason, it was not possible to identify these to generic level and thus they have been recorded as *Haemoproteus*/*Plasmodium* (Figure 2).

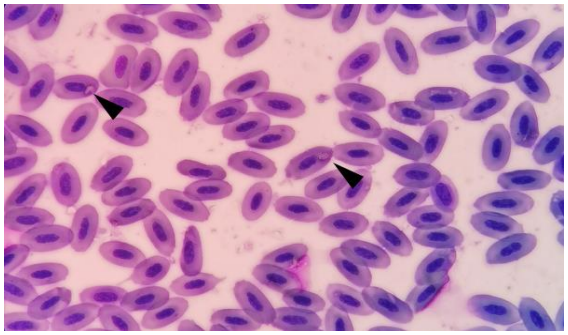

Figure 2. Ring-shaped young gametocytes in red blood cells (arrow heads). Young gametocytes are roundish to oval with an even outline, a peripheral dark nucleus and a large white vacuole.

As for the detection of *Leucocytozoon* spp., the infection was not observed in any of the 70 smears observed in the present study.

Other variables, such as sex predispositios, housing conditions of the birds and seasonal differences, were not studied. The morphological identification of the species was not performed in this study.

4. DISCUSSION

The detection of *Haemoproteus* spp. and *Plasmodium* spp. infection is more common than *Leucozytozoon* spp. infection. This difference is not because *Leucocytozoon* spp. infection is rare, but

due to the life cycle of this parasite that is detected in peripheral blood only for short periods of time (Hellgreen *et al.*, 2004). The prevalence of hemoparasites infection varies worldwide. Comparisons between studies are difficult to establish because of the differences related to the study design such as the population studied (symptomatic vs. asymptomatic; wild vs. captive), geographic differences and the use of different detection methods.

A greater diversity of these haemoparasites is related to countries with warm climates (Valkiünas & Iezhova, 2017). The dispersal of haemoparasites by migratory birds and international trade are also pointed out as agents of dissemination (Valkiünas & Iezhova, 2017). Barriers for this dispersal have been identified such as geographic, environmental and interspecies barriers (Lima, & Pérez-Tris, 2020). However, climate changes can cause an impact on haemoparasites and its vectors distribution and frequency, therefore, affecting the balance of the ecosystems (Hong *et al.*, 2021; Lima, & Pérez-Tris, 2020; Prieto-Torres, Rojas-Soto, & Lira-Noriega, 2020; Ortiz-Catedral *et al.*, 2019).

In this study microscopic observation was used as a diagnostic method. Blood smears were taken for direct microscopic observation for the identification of *Haemoproteus* spp., *Plasmodium* spp. and *Leucocytozoon* spp. Seasonal variation of haemosporidians infection would be interesting to evaluate. However, all samples collected for this study were obtained within a narrow window of time, making it impossible to analyse this factor.

Knowing that these exotic captive birds can be infected, avian veterinarians must be aware of the importance of haemosporidia infection, since the impact of haemosporidians on their hosts is difficult to interpret from a clinical perspective and foreign pathogens may cause unpredictable pathogenesis when infecting naïve hosts.

5. CONCLUSION

Given the lack of data on hemosporidian distribution in Portugal, namely in Psittacidae species, this work represents an important contribution to a better understanding of the epidemiological impact of the infection in captive fauna of *Psittacus erithacus*. To the best of our knowledge, this is the first surveillance study of haemosporidia in this species and in psittacines in Portugal. More studies on hemoparasites detection in other psittacine species would be of great interest, as well as the evaluation of the seasonal variation of haemosporidian infection.

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