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# *Haemosporidian* Parasites in Domestic and Wild Birds

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**Abstract**

*This study aims to provide brief information about the role of haemosporidian parasites in birds and significant implications for avian health, population dynamics, and control efforts. Haemosporidian parasites, belonging to the phylum Apicomplexa, are a diverse group of protozoan organisms that infect a wide range of vertebrate hosts, including birds. These parasites have a complex life cycle, with both sexual and asexual stages, and are transmitted by blood-feeding insects such as mosquitoes, midges, and blackflies. The potential impact of these parasites include a wide range of health issues in infected birds, including anemia, decreased body condition, and reduced reproductive success. Additionally, the distribution of parasitic populations within individual hosts can lead to complex interactions between the host and its parasites, which can influence the overall population structure and dynamics of both the host and the parasite. The prevalence and genetic diversity of haemosporidian parasites can also vary depending on various factors, such as the migratory patterns of birds, availability of suitable vectors, and environmental conditions in different regions. In conclusion, haemosporidian parasites play a crucial role in the dynamics of bird populations, influencing their health, fitness, and population structure. Understanding the complex interactions between these parasites and their avian hosts is essential for developing effective strategies for the conservation and management of bird populations.*

## Introduction

Avian haemosporidians is a group of parasites which distributed worldwide and belong generally to Haemoproteus, Plasmodium and Leucocytozoon (El-Ghany, 2023). It originates in past decades with various genetic diversities (>3,500 strains) that belonging to different or same morphospecies (Harl et al., 2020). Furthermore, obtaining high-quality DNAs from haemosporidians allows deeper genomic and transcriptional evaluation result in first sequencing genome of avian parasite haemosporidians, and reconstruction becomes possible to some transcripts (Bensch & Hellgren, 2020). However, the history of research into haemosporidians in birds belongs back to 1880, in which, Danilewsky discovers a common intracellular parasite similar to malaria that infected birds; and then, he classified it into Haemoproteus, Plasmodium and Leucocytozoon (Santiago-Alarcon & Marzal, 2020). Furthermore studies have been conducted to demonstrating the pathology of the parasites in birds which being similar to malaria parasite in human, and the effect of season in transmission of infection in wild birds (Valkiūnas & Iezhova, 2018). In 1897, McCallum discovers sexual process (such as flagella) in haematosporidia which infects pigeon (Perkins, 2014). Furthermore, other researchers have been studied avian malaria discovered in mosquitoes (Diptera: Mosquitoidae) and the spread of the parasite. After, it was discovered that several species of mosquitoes transmit malaria parasites to birds; avian malaria parasites were used in many human malaria experiments (Valkiūnas & Iezhova, 2018). However, with the discovery



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of haemosporidians in rodents and primates, haemosporidians in birds was an important candidate for study (Lutz et al., 2016). However, research on malaria parasites in birds continues to play important roles for advancing medical search and studying parasite stages in vitro. Furthermore, studies of avian haemosporidians over the past two decades have led to development of strategies for pathogen management and prevention (Duc et al., 2023).

Malaria was recognized in Chinese texts as early as 2700 BC and in Egyptian papyri date back to 1500 BC, with convincing evidence from around 800 to 400 BC (Norrie, 2016). However, early studies in the tropics revealed the involvement of mosquitoes in the spread of avian malaria, although haemosporidians parasites have focused on reporting various parasites (Veiga et al., 2024). Then, 62 new species from Asia (mainly India) and 48 new species from Africa (mainly South Africa) have been described (Santiago-Alarcon & Marzal, 2020).

Table 1. Taxonomic Classification of Protozoa

| Kingdom | Phylum | Class | Order | Family | Genus (Reference) |
|---|---|---|---|---|---|
| Protozoa | Myzozoa | Aconoidasida | Haemosporina | Plasmodiidae | *Plasmodium* (Lumsden & Bertram, 1940) |
| Protozoa | Apicomplexa | Aconoidasida | Achromatorida | Haemoproteidae | *Haemoproteus* (Dimitrov et al., 2014) |
| Protozoa | Myzozoa | Aconoidasida | Achromatorida | Leucocytozozoidae | *Leucocytozoon* (Valkiūnas et al., 2010) |

Many economically and medically important species of the subphylum Apicomplexa, including Plasmodium, Cryptosporidium, Toxoplasma gondii, Babesia, Theilaria, Isospora and Eimaria lack cilia and flagella except the microgametes but having aggressive organelles (rhoptries, micronemes, and polar rings) that form the apical complex (Alkefari et al., 2017; Sray et al., 2019; Al-Abedi et al., 2020; Al-Shaeli et al., 2020; Ajaj et al., 2021; Gharban & Al-Kaabi, 2022; Gharban et al., 2023). In addition, they often contain one or more mitochondria and move in a unique shape, apicoplast. In motile invasive stages (merozoites, ookinetes, and sporozoites) such as Plasmodium using specialized secretory and motile organelles resemble elongated mononuclear cells that can invade cells and migrate into tissues (Bensch and Hellgren, 2020). Merozoites form the active and smallest developmental stage of the hematosporidian, known as the invasive stage.

They enter the red blood cells through special organs without destroying them, which allows them to carry out the invasion process. Excretory organs (rods and microns) are pear-shaped spheres, two in number. Invasion is followed by the secretion of small round vesicles called dense particles, and longer vesicles called exosomes are used by merozoites to exit the schizont. There are three membranes; the plasma membrane is on the outside. During invasion, merozoites are propelled by the movement of actinomyosin and contain numerous longitudinal microtubules that help them attach to the inner layer, which also attaches to the anterior surface of the polar ring (Brown, 2000; Valkiūnas & Iezhova, 2017; Power, 2019). The genus Haemoproteus is similar to the genus Leucocytozoon. The gametocytes of some species turn into erythrocytes, others into leukocytes, but it is small red particles that distinguish the species (Haemoproteus and Plasmodium), and lacks the melanin pigment were shown in two species (Ivanova et al., 2018).

The term "malarial parasite" is debated because lack of phylogenetic understanding of this class of parasites. Traditionally, species of the genus Plasmodium are considered true


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haemosporidians (Clark et al., 2014). Plasmodium uses different Diptera insect of the family Culicoididae. Mosquitoes are mainly spread by the bites of mosquitoes of the family Ceratopogonidae; while, Haemoproteus is spread by Ixodes flies (Santiago-Alarcon et al., 2012).

**Economic and health Importance**

***Effect of haemosporidian infection on avian hosts***

It was long thought that bird haemosporidians was not pathogenic to hosts in its natural habitat, because infected animals did not show clinical signs (Donovan et al., 2008). Other reports detected the effects of infection on birds ranging from reduced growth to death (Ellis et al., 2020). It should be noted that chronically infected birds act important roles as reservoir of the parasite, allowing subsequent infection of susceptible hosts. Chronically infected hosts may suffer from hyperparasitemia during the breeding season, when herd immunity wanes (Ferraguti et al., 2020). Marzal et al. (2005) studied the effect of Haemoproteus spp. on reproductive success of migratory house martin by treating birds with antimalarial drug (primaquine) and monitor breeding success. They showed that the treated birds showed a 39% and 42% increase in egg hatching and fledgling success, respectively, as well as an 18% increase in egg hatching and fledgling success. Knowles et al. (2010) used an antimalarial drug to treat blue tits to study the effect of chronic malaria infection on the health of blue tits. They found that treated blue tits (Cyanestes caeruleus) improved nesting and nesting success. Marzal et al. (2005) showed Haemoproteus spp. infections were reduced survival (Delichonerbica). Infected birds with high infection rates tend to be weaker. Other studies of haemosporidians bird parasitology have used mist nets to capture wild birds, but this is biased because birds captured in this way are generally active, healthy, and asymptomatic. Therefore, available figures indicating prevalence in the wild are likely much higher than reported.

***Economic and health importance***

Host survival and fitness can be affected by parasitism, while the host population and distribution of these parasites negatively impact survival, host condition, and reproductive success (Pigeault et al., 2018). Avian malaria is widespread economically and globally, and microorganisms such as P. gallinaceum, and P. dure are widespread. Nuclear proximity is of great importance to the poultry industry and can cause up to 90% mortality among birds (Tembe et al., 2023). In general, Plasmodium spp. considered more virulent than Haemoproteus, both infections cause damage to the host (Dimitrov et al., 2015). In birds, reproductive costs can be controlled by the allocation of energy between reproduction and defense against parasites, but the establishment of parasites in the host reduces the energy resource (Esteban Henao, 2019). Avian malaria is a potential threat to both wild birds and domestic poultry, and Plasmodium is a pathogen of poultry in South America, Asia and Africa (Bonneaud et al., 2009). It has been suggested that the prevalence of P. gallinaceum avian malaria in different areas of Thailand affected the egg and poultry production industries, while others suggested that the increase in parasite density was due to different effects (Sehgal, 2015; Boonchuay et al., 2023).

Avian haemosporidians play an important role in human malaria and serve as a model for this study, encouraging the development of medical parasitology (Marzal, 2012). Several researchers mentioned that the models have been approved, including lifecycle studies, in vitro culture development, chemotherapy, and vaccines (Vinetz et al., 2011; Delves et al., 2012; Richie et al., 2015). Furthermore, inexpensive avian models of Plasmodium spp. can be used in any laboratory, especially for immunological (biochemical and genetic) studies. In both eras,


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haemosporidians biology and evolution in bird populations are attracted (Antonelli et al., 2020). Avian malaria is unlikely to infect humans and does not mutate in mammalian hosts (Escalante et al., 2022).

**Epidemiology**

In biological communities, diversity of Haemosporidia follows climatic alteration that considered important factors in protozoan transmission. Sehgal (2015) mentioned that climatic factor directly and indirectly influences vector abundance and resulting parasites in communities. Habitat fragmentation and development can result in creating more open spaces and improving vector distribution. Insect abundance can affect parasites both directly and indirectly by affecting the timing of vector development (van Hoesel et al., 2020). A recent study found that most of the captured mosquitoes are considered main vectors to transmission of malaria parasites; while, mosquito prevalence is related to changes in rainfall (Veiga et al., 2024). The salinity of water has also been identified in incidence of infectious disease, and researchers believe that mosquito prevalence is due to a delay in the response to rainfall due to changes in rainfall patterns (Krama et al., 2015). The sensitivity of vector to environmental conditions varies, with more sensitive vectors occurring less frequently in arid environments or areas with strong seasonal variations in climate, which subsequently influence the spread of the parasite (Chapa-Vargas et al., 2020).

Temperature is considered an important in spreading of a parasite and vectors and in metabolic rate. The parasite takes up to 14 days and shows significant variations depending on ambient temperature, which affects transcription success (Álvarez-Mendizábal et al., 2021). Because risk of vector mortality is very high during this period, it may be beneficial for parasites to manipulate vector behavior to increase evasion by vector appetite after initial ingestion of blood (Cornet et al., 2019). Various researchers showed that hematosporidians are unable to predict historical characteristics of parasites or mosquitoes in response to temperature changes assume a continuous linear response that is unrealistic to observe (Mora-Rubio et al., 2023). The parasite transmission temperature is 25°C, which leads to very different estimates under current climate change trends of the prevalence and spread of the parasite. Temperature impacts include seasonal changes, insect metabolism results in longer periods within season breeding events (Mordecai et al., 2013).

In tropical areas, parasite availability varies depending on seasonal climate and reproductive cycle of the host. During winter, most birds have very few or no parasites in their blood, and the infection can remain hidden leaving very few parasites in blood of most birds. Their blood maintains a chronic infection (Van Hemert et al., 2019). Temperatures increase the vector emerges and multiplies, and once host birds begin to breed, the chance of infecting new individuals, mainly young birds that have never been infected before, are increased significantly (Ellis et al., 2019). Furthermore, reproductive activity requires energy, and it has been repeatedly shown that the interplay between energy and immune function can lead to the recurrence of chronic infections. Therefore, seasonal variations influence protozoan communities (Hahn et al., 2018). In tropical habitats, these had large seasonal variations with little change in climate and lowland forests, while the two habitats with the largest changes had parasites hosted by more common species (Ferreira Junior et al., 2017). Generally, haemosporidians parasite species likely infect a variety of hosts and can spread rapidly among harmless populations through host switching processes. This suggests that climate change, such as changes in temperature and precipitation, as well as more severe seasonal variations, may increase the spread of parasites in local communities (Santiago-Alarcon et al., 2019). Relative abundance of effective vectors and susceptible hosts are environment determining factors.



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Parasites can destabilize or disrupt host communities with multiple parasite lineages per species (Atkinson, 2023). In general, the environmental conditions, distribution of haemosporidians are more favorable for vector survival and reproduction in some areas and less favorable in others, and are expected to vary equally (Padilla et al., 2017; Chapa-Vargas et al., 2020).

**Pathogenesis**

In comparison, species of the genera Plasmodium and Leucocytozoon are considered more pathogenic, but some Hemoproteus species have been reported to cause disease in birds. Infection in birds involves the following main phases: (a) an incubation period, during which the parasite first appears in the internal organs, (b) an acute period, during which the parasite appears in the bloodstream and is detected on blood smears, (c) crisis, when parasites reach their peak. Often the parasite persists for several years or even a lifetime, and the parasite can reproduce in the blood under conditions that place high demands on the host (Ilgūnas et al., 2016; Schumm et al., 2019). Even parasites of the same species can cause very different pathological effects. Several studies have shown that serious effects such as severe anemia, pneumonia, thermoregulation disorders, muscle cysts or brain damage are associated with specific types of parasites during the latent or acute phase of blood cell infection. This effect is enhanced when an infected host first encounters the parasite during primary infection (Atkinson & Van Atkinson, 1991).

Although the virulence of Plasmodium species depends on their strain and host susceptibility, the impact of Plasmodium infection on bird health is generally relatively minor (Gupta, 2024). Merozoites follow a multi-step process in which they first enter the red blood cells, and then attach to the outer membrane of the red blood cells and then the tips of the merozoites come into contact with the surface of the red blood cells and are induced to produce red blood cells (Jhun, 2018). Birds might be infected through tight junctions and is then secreted by the rhomboids, forming a deep layer in the lining of the fossa. Adhesions are complex chemical mixtures secreted by micronemes and merozoite rods, including proteases and membrane modifiers. The interactions between them include the capture of red blood cells; the formation of junctions and the production of parasitic fluids involved in cyst formation allow parasites to enter. Selective uptake by host cells requires interaction between receptors located on the surface of merozoites and ligands located on the membrane of red blood cells. In fact, the parasite can produce multiple receptors that are accepted by different ligands of the host cells (Brown, 2000; Ouologuem, 2014). It showed that merozoite apical antigen, another secreted micronemal protein, involved in apical junction formation, and merozoite envelope proteins are also important for binding to red blood cells. Once the protein-1 lose their invasive properties and are taken up into the cytoplasm of the red blood cells, where they are proteolyzed. The parasite turns into a trophozoite, which feeds the very hungry, and the hemoglobin test product in the food vacuole is converted into an insoluble malarial pigment (plasmazoin), which accumulates in a large central vacuole containing the pigment (Martinez, 2013; O'Donoghue, 2017). Hemoglobin supplies most of the amino acids (arginine, methionine, isoleucine) required for parasite growth through proteolysis and is also essential in plasma. Fatty acids are synthesized by the malaria parasite, and although the parasite is highly dependent on external sources, the synthesis of fatty acids appears essential to development of malaria (Galen et al., 2015; Delhaye et al., 2018).

Circulating sporozoites enter the liver via protective vascular endothelial layers can pass through multiple hepatocytes before entering the hepatocytes. It feeds on hepatocytes and proliferates to form paratrophocytes and erythrocyte schizoites, producing hundreds of merozoites. Merozoites become trapped (clustered) in mitochondria (membrane-covered



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packages) in the bloodstream, enter the pulmonary circulation, affect small alveolar blood vessels, and die when the mitochondrial membrane ruptures and matures. Merozoites are released into the bloodstream long after the initial infection (Duc, 2023). Depending on the route of infection, intramuscular or subcutaneous, intravenous or intramuscular, more severe infections are associated with a higher risk of disease, which involves clinical symptoms, death and organ pathology (Yoshimoto et al., 2021). As a result, the severity increased number of parasites that survive injection into the host and parasite development can vary considerably within and between experiments. In addition, there are potential sources of variation between experimental studies (age, sex, breed, reproduction, and diet), (Valkiūnas & Iezhova, 2017). Damage to capillaries and other brain cells can lead to neurological symptoms such as altered mental status, balance problems and blindness. Injuries can cause intraocular bleeding and blindness (Duc et al., 2023). Infected vascular endothelial cells can block micro-vessels and cause thromboembolism, and capillary damage can also lead to bleeding (Himmel et al., 2024). In addition to circulating leukocytes and red blood cells, Leucocytozoon also affect tissue macrophages and endothelial cells, which subsequently form creates large tissue schizonts up to 700 µm in diameter (Desser, 2012). They are more common in the spring, when lightning is present, and the host responses to infection include multiple necrotic lesions in liver, spleen and kidneys, and when these organs are attacked, atrophy of the heart and skeletal muscle, which can lead to pulmonary infection (Donovan et al., 2008). During inflammation, monocytes and lymphocytes are produced and the trophic response is reduced. Birds that develop hemolysis may develop significant hemosiderosis in the spleen and liver (Ewbank, 2016).

**Clinical signs**

Avian malaria infections related to parasitemia, leads to anemia and reduced motility in the acute phase. After, the parasite generally becomes stable at low levels (the so-called chronic phase), and some Plasmodium species may disappear completely from the bloodstream and remain inactive in the asexual phase (Emmenegger, 2018; Gozalo et al., 2024). Replication after high replication cycles or long incubation periods, is often accompanied by high levels of stress, such as in the context of malnutrition or energy-intensive activities such as reproduction or migration, where host immune function is often suppressed (Cornet et al., 2014). The dormancy of haemosporidians parasites in poultry is thought to be an adaptive mechanism that allows parasite survival. In this case, the parasite must be able to survive in its current host for a long time without causing death (Pigeault et al., 2018).

Infection with the parasite species Plasmodium and Haemoproteus remains very persistent for many years, while infection with the parasite species Leucocytozoon (Santiago-Alarcon & Marzal, 2020). Because haemosporidians parasites can persist in all avian hosts, these parasites can accumulate throughout an individual's life, even if the costs of a single reproductive effort are small or negligible. As a result, lifetime reproductive success is reduced. Population-wide effects can occur if the fertility of an entire population is limited. This potential impact on reproduction could endanger the survival of endangered species (Rooyen et al., 2013; Pigeault et al., 2020).

**Diagnosis**

Laboratory and field methods for the study of haemosporidians protozoa begin with the microscopic examination and molecular techniques to detect infection by PCR and rapid sequencing. Different studies have been focused on combining multiple methods can better estimate the true distribution of hematosporidian, especially their distribution and biodiversity (Rodrigues et al., 2020). Although these parasites have been studied for more than 100 years,


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there is no optimal method to determine the infection status since neither microscopy nor PCR are fully adequate to detect low-level erythrocytic infections and do not ignore latent infections confined to pre-erythrocytic in deeper tissues (Poblete et al., 2024). Therefore, false negative tests may occur more often than expected (Ishtiaq et al., 2017). Furthermore, the intensity of infection by arthropod vectors can vary considerably, making it difficult to distinguish between vegetative infection limited to blood meals and successful spore development and salivary gland invasion by sporozoites (Burkot & Graves, 2000). Detecting pseudo-infection in vertebrate hosts with PCR is also difficult, because amplification of parasite DNA cannot distinguish between sporozoite and asexual stages of the parasite (Valkiūnas et al., 2014). In field, allowing PCR to be used to amplify and sequence the nuclear and mitochondrial genes of parasites, allowing the lineage of individual parasites to be determined for the first time and assign species. Molecular methods have several advantages. Furthermore, PCRs are unable to differentiate duplicate infections with haemosporidians of the same subtype. Some of these issues can be addressed by using high-throughput sequencing methods to identify specific lineage populations (Bensch & Hellgren, 2020; Musa, 2023). However, this approach is more important, especially when this method is used in unknown geographical areas or on new parasites with unknown parasite morphology and genetic diversity, the well-known bird morphospore (Nourani et al., 2018).

Microscopy and molecular methods when a combined, it important and increasingly common for the diagnosis of hematosporidial infections, offering significant advantages over the use of either method alone (Bensch & Hellgren, 2020). The combined approach increases detection sensitivity and allows the association and potential identification of specific traits associated with the host or life history morphism. It is also not possible to determine the (acute) stage of the infection based on blood tests alone (Huang, 2021). Although information on the incubation period and recovery from previous infection is not essential for epidemiological research on haemosporidians parasites, it is of great importance to determine the infection status (Ganser et al., 2020). In vertebrate hosts, antibody titers can be used to detect low-intensity chronic antibodies (Zhang et al., 2022). Serological techniques have been used in wild and captive penguins, native Hawaiian forest birds; however, this method is not generally applicable to different host-parasite associations (Ings & Denk, 2022).

## Treatment

Treatment of Plasmodium infection in birds is limited to commercially available or approved antimalarials. However, some drugs, such as chloroquine and doxycycline, or artesunate and tafenoquine, have been experimentally shown to be effective in reducing parasitemia and infection (Pugliese et al., 2023). The extraerythrocytic status of the parasite can lead to persistent parasitemia and recurrence, and treatment success rates vary between cases and herds. In addition, the treatment of diseases in animals is far from ideal, and pyrimethamine and sulfamethoxine are sometimes used as prophylactic agents. Several compounds have also been tested experimentally including primaquine, desipramine hydrochloride, sulfaquinoxaline, and ketotifen (Chapman & Rathinam, 2022). In addition, the disease has been treated with chemotherapy combinations such as pyrimethamine-sulfadoxine and mefloquine, atovaquone/proguanil to treat avian malaria in canaries, other sick birds, penguins and birds of prey (Cortez-Maya et al., 2020). In general, the effectiveness of antimalarials in the treatment of wild birds is limited due to difficulties in administration and the possibility of development of drug-resistant parasites (García-Longoria et al., 2016).

## Control


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Vaccines and antibiotics have been used successfully to protect people and pets from infectious diseases. Most wildlife vaccines target mammalian hosts of zoonotic diseases, but developing vaccines against protozoan pathogens has been difficult (Palatnik-de-Sousa & Nico, 2020; Florin-Christensen et al., 2021). Birds were one of the first experimental models to develop a vaccine against the malaria parasite, but vaccination of wild bird populations faces major challenges (Simwela & Waters, 2022). Several experimental vaccines have been used, including synthetic vaccines based on UV-irradiated sporozoites, merozoites, gametes and parasite surface molecules (Viljoen et al., 2021). DNA vaccines based on perisporozoite proteins from P. gallinaceum and P. relictum have been shown to protect against P. relictum in penguins and canaries, but the duration of immunity is short and the birds become infected after 1 year (Zhang et al., 2022). Understanding the natural process of spore development can improve malaria prevention strategies. Infections in captive birds can be avoided by building fly enclosures, but it is virtually impossible to prevent infections in wild birds. Preventing the transmission of vector is one of the most important steps in the elimination of malaria, and many environmental factors, entomological characteristics and especially the genetic background (species and strain) of the vector influence the transmission of malaria (Rasidi & Cornejo, 2021). Vaccination has long been considered the best way to control livestock diseases and has been shown to be safe for both animals and consumers; however, some of these antibodies could be used in host defense or against invading organisms (de Wit & Montiel, 2022).

## Conclusion

Haemosporidian parasites play a crucial role in the dynamics of bird populations, influencing their health, fitness, and population structure. Understanding the complex interactions between these parasites and their avian hosts is essential for developing effective strategies for the conservation and management of bird populations.

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