SHORT COMMUNICATION

A morphological survey of avian Haemosporida parasites in Colombian wild birds

Un estudio morfológico de Haemosporida aviar en aves silvestres colombianas

Um levantamento morfológico de Haemosporida em aves selvagens da Colômbia

Arley Onasis Arroyave Pérez[1]; Analorena Cifuentes-Rincón[2,3]; Ingrid Johana Roldán Carvajal[4]; Nathalia M Correa-Valencia[5*].

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***Corresponding author:** Nathalia M Correa-Valencia. Calle 70 No. 52-21. Phone number: (+57) 301 4243408. Email: mariadelp.correa@udea.edu.co

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[1]School of Veterinary Medicine, Faculty of Agricultural Sciences, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia.
[2]IMPRONTA research group, Universidad Cooperativa de Colombia, Ibagué, Colombia.
[3]Department of Fish and Wildlife Conservation, Virginia Tech, Virginia, United States.
[4]HEMO research group, School of Microbiology, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia.
[5]CENTAURO research group, School of Veterinary Medicine, Faculty of Agricultural Sciences, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia.

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Abstract

Introduction: Infection by protozoan hemoparasites causing malaria in birds can lead to physical, reproductive, and behavioral alterations, resulting in a decline in the populations of affected wild birds. **Objective:** To identify the presence of parasite agents causing avian haemosporidioses, including malaria (caused by *Plasmodium*) and other closely related species (i.e., *Haemoproteus*, *Leucocytozoon*) in a wild bird population in the municipality of Jardín (Antioquia, Colombia), through blood smears and to explore the bird-level factors associated with positivity. **Methods:** A descriptive cross-sectional study was conducted with non-probabilistic convenience sampling. Blood samples were obtained from wild birds captured with mist nets. Each bird was characterized according to its genus, species, sex, and age group. Three blood smears per bird were prepared and examined for parasites belonging to *Haemoproteus*, *Leucocytozoon*, and *Plasmodium* genera. Descriptive statistics and the association between study variables and the dichotomous outcome of blood smear analysis (positive or negative) using Fisher's exact test. **Results:** A total of 46 wild birds belonging to 20 different species of the orders Passeriformes and near-Passeriformes were captured at six different locations in the study municipality (between 1,665 and 2,053 m.a.s.l.) in July 2022. Parasites belonging to Haemosporida, including those causing avian malaria were found, with an overall infection prevalence of 34.8% (16/46). Among the birds, 32.6% (15/46) were positive for *Plasmodium* sp., 6.5% (3/46) for *Haemoproteus* sp., and 4.3% (2/46) for *Leucocytozoon* sp. In addition, 6.5% (3/46) of the birds were coinfected with 2 or 3 parasites belonging to different genera. An association was found between *Plasmodium* sp. infection and age group (p=0.050). **Conclusion:** This study contributes to the knowledge about hemoparasites in wild birds in Colombia, reporting the presence of agents of avian malaria, *Haemoproteus* and *Leucocytozoon* in the study area. Further research is required on the molecular identification of protozoan hemoparasites, pathogenicity, the health status infected birds, and the attributable impact on their populations.

Keywords: *avian malaria; blood smear; <u>Haemoproteus</u>; Haemosporida; <u>Leucocytozoon</u>; <u>Plasmodium</u>; protozoan; wild bird.*

Resumen

Introducción: La infección por hemoparásitos protozoarios que causan malaria en aves puede generar alteraciones físicas, reproductivas y de comportamiento, lo que conlleva a una disminución en las poblaciones de aves silvestres afectadas. **Objetivo:** Identificar la presencia de agentes parasitarios causantes de hemosporidiosis aviar, incluida la malaria (causada por *Plasmodium*) y otras especies estrechamente relacionadas (i.e., *Haemoproteus, Leucocytozoon*), en una población de aves silvestres del municipio de Jardín (Antioquia, Colombia) mediante frotis sanguíneos y explorar los factores asociados con la positividad a nivel individual en las aves. **Métodos:** Se realizó un estudio descriptivo transversal con un muestreo no probabilístico por conveniencia. Se obtuvieron muestras de sangre de aves silvestres capturadas con redes de niebla. Cada ave fue caracterizada según su género, especie, sexo y grupo etario. Se prepararon y examinaron tres frotis sanguíneos por ave para detectar parásitos de los géneros *Haemoproteus*, *Leucocytozoon* y *Plasmodium*. Se emplearon estadísticas descriptivas y la asociación entre las variables del estudio y el resultado dicotómico del análisis del frotis sanguíneo (positivo o negativo) mediante la prueba exacta de Fisher. **Resultados:** En total, se capturaron 46 aves silvestres pertenecientes a 20 especies diferentes de los órdenes Passeriformes y cercanos a Passeriformes en seis localidades del municipio de estudio (entre 1.665 y 2.053 m.s.n.m.) en julio de 2022. Se hallaron parásitos pertenecientes a Haemosporida, incluidos los causantes de la malaria aviar, con una prevalencia general de infección del 34,8% (16/46). Entre las aves, el 32,6% (15/46) fueron positivas para *Plasmodium* sp., el 6,5% (3/46) para *Haemoproteus* sp., y el 4,3%

(2/46) para *Leucocytozoon* sp. Además, el 6,5% (3/46) de las aves estaban coinfectadas con 2 o 3 parásitos de diferentes géneros. Se encontró una asociación entre la infección por *Plasmodium* sp. y el grupo etario (p=0,050). **Conclusión:** Este estudio contribuye al conocimiento sobre hemoparásitos en aves silvestres en Colombia, reportando la presencia de agentes causantes de malaria aviar, *Haemoproteus* y *Leucocytozoon* en la zona de estudio. Se requiere mayor investigación sobre la identificación molecular de hemoparásitos protozoarios, su patogenicidad, el estado de salud de las aves infectadas y el impacto atribuible a sus poblaciones.

Palabras clave: *ave silvestre; frotis sanguíneo; Haemoproteus; Haemosporida; Leucocytozoon; malaria aviar; Plasmodium; protozoo.*

Resumo

Introdução: A infecção por hemoparasitas protozoários que causam malária em aves pode gerar alterações físicas, reprodutivas e comportamentais, levando a uma diminuição nas populações de aves silvestres afetadas. **Objetivo:** Identificar a presença de agentes parasitários causadores de hemosporidioses aviárias, incluindo malária (causada por *Plasmodium*) e outras espécies intimamente relacionadas (i.e., *Haemoproteus, Leucocytozoon*), em uma população de aves silvestres no município de Jardín (Antioquia, Colômbia) por meio de esfregaços sanguíneos, e explorar os fatores associados à positividade a nível individual nas aves. **Métodos:** Foi realizado um estudo descritivo transversal com amostragem por conveniência não probabilística. Amostras de sangue foram obtidas de aves silvestres capturadas com redes de neblina. Cada ave foi caracterizada de acordo com seu gênero, espécie, sexo e grupo etário. Foram preparados e examinados três esfregaços sanguíneos por ave para detectar parasitas dos gêneros *Haemoproteus, Leucocytozoon* e *Plasmodium*. Foram empregadas estatísticas descritivas e a associação entre as variáveis do estudo e o resultado dicotômico da análise dos esfregaços sanguíneos (positivo ou negativo) utilizando o teste exato de Fisher**. Resultados:** No total, foram capturadas 46 aves silvestres pertencentes a 20 espécies diferentes das ordens Passeriformes e próximas a Passeriformes em seis localidades do município de estudo (entre 1.665 e 2.053 m.s.n.m.) em julho de 2022. Parasitas pertencentes à ordem Haemosporida, incluindo os causadores da malária aviária, foram encontrados, com uma prevalência geral de infecção de 34,8% (16/46). Entre as aves, 32,6% (15/46) foram positivas para *Plasmodium* sp., 6,5% (3/46) para *Haemoproteus* sp. e 4,3% (2/46) para *Leucocytozoon* sp. Além disso, 6,5% (3/46) das aves estavam coinfectadas com 2 ou 3 parasitas de diferentes gêneros. Foi encontrada uma associação entre a infecção por *Plasmodium* sp. e o grupo etário (p=0,050). **Conclusão:** Este estudo contribui para o conhecimento sobre hemoparasitas em aves silvestres na Colômbia, relatando a presença de agentes causadores de malária aviária, *Haemoproteus* e *Leucocytozoon* na área de estudo. Mais pesquisas são necessárias sobre a identificação molecular de hemoparasitas protozoários, sua patogenicidade, o estado de saúde das aves infectadas e o impacto atribuível em suas populações.

Palavras-chave: *ave silvestre; extensão sanguínea; Haemoproteus; Haemosporida; Leucocytozoon; malária aviária; Plasmodium; protozoário.*

Introduction

Protozoan hemoparasites of the order Haemosporida affect various animal groups, such as mammals, reptiles, and birds. The agents responsible for transmitting avian haemosporidioses among wild bird populations belong to the genera *Plasmodium*, *Leucocytozoon*, *Fallisia*, and *Haemoproteus* (Villalva-Pasillas *et al.*, 2020). These agents can be transmitted by vectors such as mosquitoes, hippoboscid flies, *Culicoides* species and simuliids, which when present in areas with high availability, variety of hosts, or with low bird diversity —for example in high latitudes, allow the parasite transmission (Merino *et al.*, 2008; Ventim *et al.*, 2012).

Vector-transmitted hemoparasites that cause haemoproteoses, including avian malaria, can lead to physical, reproductive, and behavioral damage in birds. During periods of immunosuppression, infections that are subclinical may increase in parasitemia, decreasing survival and reproductive capacity (Braga et al., 2011). Hemoparasite infections often present as a chronic stage, and their dynamics are potentially influenced by coinfections, which can increase mortality and reduce the populations of affected birds (Astudillo *et al.*, 2013). Blood parasites have been associated with increased stress levels, increased susceptibility to diseases, and reduced survival rates in infected birds (Levin and Parker, 2012; Astudillo *et al.*, 2013; López-Serna *et al.*, 2021).

In addition, these hemoparasites pose a risk to territorial ecology, related production systems, and bird populations, potentially leading to extinction of some species, which negatively impacts strategic ecosystems (Levin and Parker, 2012; Chan *et al.*, 2013; Naqvi *et al.*, 2017; Young *et al.*, 2017). Nevertheless, parasites are important part of the biodiversity. That is, a rich diversity of parasites is expected in regions with high bird and ecosystem diversity (Moore *et al.*, 2023). Therefore, it is important to review the distribution of these pathogens in wild bird populations to advance conservation strategies, prevent the increase and spread of pathogens that could reduce wild populations and contribute to the extinction of threatened species (Levin and Parker, 2012; IUCN, 2020).

Considering anthroponosis and zoonosis possibilities, economic losses associated with poultry production can also be observed. Therefore, understanding infectious diseases that directly affect wild birds can support the establishment of preventive measures and facilitate the diagnosis of diseases in poultry populations (Chan *et al.*, 2013; Naqvi *et al.*, 2017). Consequently, this study aimed to identify the presence of parasite agents causing avian haemosporidioses, including malaria (*Plasmodium*) and other closely related species (i.e., *Haemoproteus, Leucocytozoon*) in a wild bird population in the municipality of Jardín (Antioquia, Colombia) through blood smears and to explore the bird-level factors associated with positivity.

Materials and Methods

Ethical considerations

The handling and collection of samples from the birds were carried out in compliance with Law 84 of 1989, which adopts the National Animal Protection Statute, creates offenses, and regulates the relevant procedures and competencies, and Decree 1376 of 2013, which regulates the permit for the collection of specimens of wild species of biological diversity for noncommercial scientific research purposes. In addition, this study received the approval of the Ethics and Bioethics Committee for Animal Experimentation of the Universidad Cooperativa de Colombia (Bioethical Concept No. BIO293, Act No. 21-109 of April 28, 2022), as part of the project "Molecular detection of avian malaria in wild birds of Southwest Antioquia and the upper Magdalena valley," regarding the capture and handling of animals, as well as the collection of samples. Similarly, the ANLA granted the Universidad de Antioquia the "Framework permit for the collection of specimens of wild species of biological diversity for noncommercial scientific research purposes" (Resolution 0524 of May 27, 2014, and Resolution 1461 of December 3, 2014), which covers this research approach.

Study area, bird sampling, and blood smear analysis

A descriptive cross-sectional study was conducted using no probabilistic convenience sampling in the geographical area of interest. Wild birds were captured in cloud forests at an altitude of 1,768 meters above sea level, with temperatures ranging from 20-25°C and humidity between 70 and 98% in the municipality of Jardín (Southwest Antioquia, Colombia) (Figure 1), a natural area corresponding to a tropical montane cloud forest, over 2 weeks (July 17 to July 30, 2022).

Capture processes followed the methodologies proposed by Ralph *et al.* (1996) and Villareal *et al.* (2004), utilizing field capture using monofilament nylon mist nets, denier size 10-12, 9-12×2.5 m, with a mesh size of 15 mm, 5 panels, and 6 shelves, designed for capturing birds and bats. The nets were operated for approximately 5 hours/net/day (6:30-11:30 a.m.). After capturing, the birds were transported to the banding station in capture bags measuring 25×30 cm, made of linen and round cord, with thick 75-gauge thread and a resistant and breathable French seam, where the birds remained for a maximum of 20 minutes post-capture.

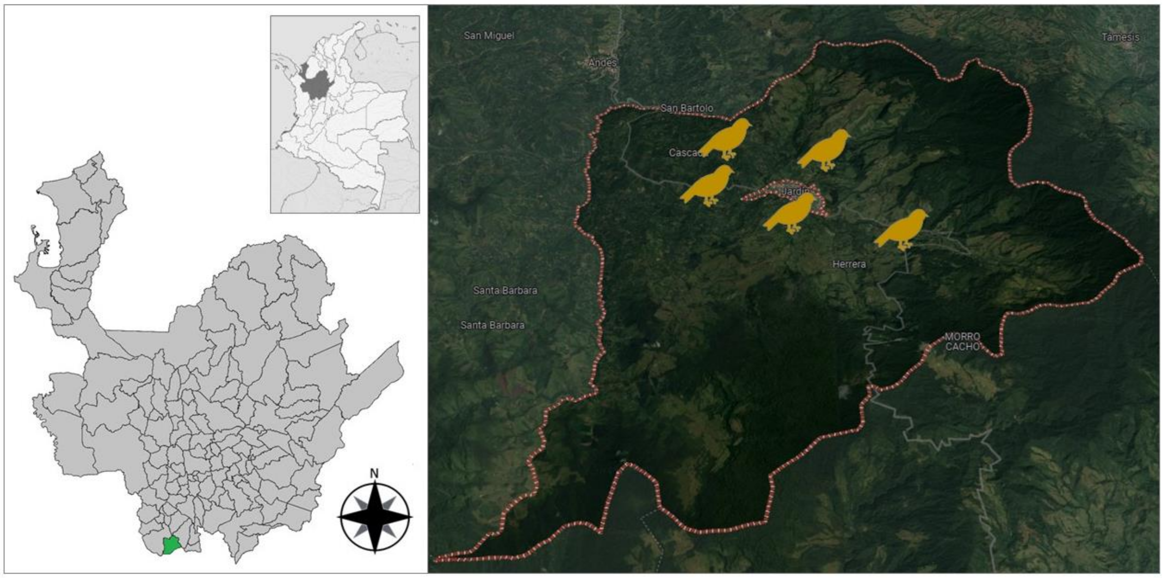

Figure 1. Location of sampling sites (bird images) inside the municipality of Jardín. Interrupted line framing (Antioquia Province, Colombia).

Subsequently, each bird was characterized (i.e., species, sex, age group) using field identification methodology based on Birds of Colombia, according to Hilty and Brown (2021), and the Classification of South American bird species for taxonomy according to the guidelines of the South American Classification Committee (Remsen *et al.*, 2022). In addition, there was an ornithological professional accompaniment for gender and species identification based on a photographic record of each captured bird.

The samples were obtained only from birds weighing more than 15 g (to minimizing stress and harm to smaller birds, to ensure sufficient blood volume and sample quality), primarily but not exclusively from the order Passeriformes. Blood samples were collected from the brachial vein of the left-wing following cleaning with antiseptic alcohol. The puncture was made using 1.0 to 1.5 mm depth sterile lancets at a 45° angle, and the blood was collected in heparinized microhematocrit tubes. Once the collection was complete, hemostasis with cotton swaps was achieved at the puncture site, and the area was cleaned to prevent predation, by removing blood odor. For future recapture identification and to avoid resampling, a small cut was made in the tail feathers. Once verified that each bird was in good condition to fly, it was released and returned to its habitat at the same place of capture.

Three blood smears were prepared for each bird, as soon as possible (under field conditions), after the withdrawal of the blood. Smears were subjected to a drying process in the field with the help of a portable fan; finally, they were placed in a plastic Coplin box and fixed in methanol for 5 minutes in the field. Once dry, the fixed smears were stored and subsequently placed on a staining rack with Giemsa (pH 7.2) for 45 minutes as previously reported under the same sampling conditions (González *et al.*, 2014; Matta *et al.*, 2024). The stained smears were properly labeled with consecutive numbers, stored in slide boxes, and transported to the Special Parasitology Laboratory at the Faculty of Agricultural Sciences (Universidad de Antioquia), where the microscopic examination was conducted.

Each blood smear was microscopically examined in search of parasites of the *Haemoproteus*, *Leucocytozoon*, and *Plasmodium* genera. Initially, low magnification (400X) was used for approximately 10 minutes; subsequently, high magnification (1000X) was used for at least 20 minutes. The reading of slides was performed by two independently trained observers (AOAP, IJRC), following identification keys and reference bibliography (González *et al.*, 2015; Lotta *et al.*, 2016; Valkiūnas *et al.*, 2018; Valkiūnas and Iezhova, 2018; Villalva-Pasillas *et al.*, 2020; Matta *et al.*, 2024).

Statistical analysis

All data generated during the study were entered into Excel spreadsheets (Microsoft Corp., Redmond, WA, USA) and then exported to Stata v.18 (StataCorp, 2023, College Station, TX, USA) for statistical analysis. Descriptive statistics were calculated for all demographic variables of interest (i.e., species, sex, age group). The associations between these variables and the dichotomous outcome of taxonomic analysis on blood smears (positive or negative) in general terms (neither positive to any of the three microorganisms of interest) and by each one were analyzed by Fisher's exact test when the tables were larger than 2×2; otherwise, chi-square test p values were calculated (significance level of $p<0.05$). In addition, Cohen's kappa coefficient was estimated between observers.

Results

A total of 46 wild birds of the Passeriformes and near-Passeriformes orders were captured at six different locations within the study municipality at altitudes ranging from 1,665 to 2,053 m.a.s.l. There were 20 different bird species recorded, with 17 adults, six juveniles, and four immatures (when possible, to define the age group), as well as eight males and three females (when possible, to define the sex). No bird was recaptured.

Parasites of the order Haemosporida causing avian 246 haemoproteoses, including malaria were found, with an overall infection prevalence of 34.8% (16/46) (Table 1). A total of 32.6% (15/46) of the birds tested positive for *Plasmodium* sp., 6.5% (3/46) for *Haemoproteus* sp., and 4.3% (2/46) for *Leucocytozoon* sp. (Figure 2). In addition, 6.5% (3/46) of the birds were coinfected by 2 or 3 parasites, belonging to different genera (i.e., an inmature *Thraupis episcopus* with *Plasmodium* sp. and *Haemoproteus* sp., and two *Stilpnia vitriolina*, one with *Plasmodium* sp. and *Leucocytozoon* sp. and the other one affected with the three parasites). Cohen's kappa coefficient between observers for blood smear examination was 0.78 (substantial agreement; 95%CI = 0.59-0.89).

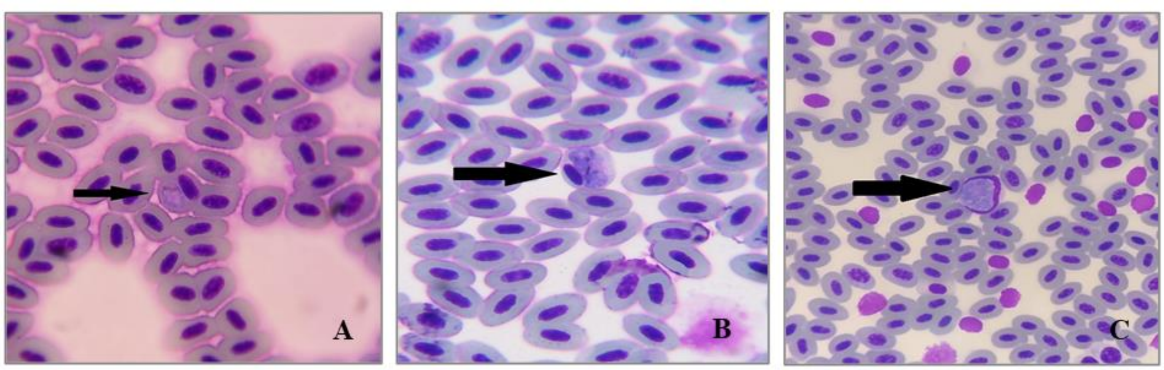

Figure 2. (A) *Plasmodium* sp. (1000X); **(B)** *Haemoproteus* sp. (1000X); **(C)** *Leucocytozoon* sp. (400X), respective findings in blood smears from avifauna samples collected in Jardín, Antioquia. Arrows indicate parasites. Giemsa stain.

The positive birds were adults and juveniles of the species *Zonotrichia capensis* (Rufous-collared Sparrow), *Thraupis episcopus* (Blue–gray Tanager), *Atlapetes albinucha* (White-naped Brushfinch), *Tangara arthus* (Golden Tanager), *Stilpnia vitriolina* (Scrub Tanager), and *Tangara gyrola* (Bay-headed Tanager), captured at altitudes between 1,655 and 2,034 m.a.s.l. Table 1 presents the characterization of the 46 study birds and the results of microscopy for the Haemosporida of interest. Infection by *Plasmodium* sp. was found to be associated with age group (p=0.050). However, we did not find other significant differences for any of the associations explored.

Table 1. Characterization of the 46 individuals and the results of microscopic examination for the Haemosporida parasites in the municipality of Jardín (Antioquia Province, Colombia).

Cons	Altitude (m.a.s.l)	Host species data				Microscopy results			
		Scientific name	**Local name (in Spanish)**	**Sex**	**Age group**	**General results**	***Plasmodium* sp.**	***Leucocytozoon* sp.**	***Haemoproteus* sp.**
1	1,655	*Sporophila nigricollis*	Espiguero capuchino	Male	Adult	Negative	Negative	Negative	Negative
2		*Myiozetetes similis*	Mosquero	ND	Immature	Negative	Negative	Negative	Negative
3		*Zonotrichia capensis*	Gorrión de montaña	ND	Adult	Positive	Positive	Negative	Negative
4		*Zimmerius chrysops*	Atrapamoscas caridorado	ND	Adult	Negative	Negative	Negative	Negative
5		*Stilpnia vitriolina*	Tángara rastrojera	ND	Adult	Negative	Negative	Negative	Negative
6		*Thraupis episcopus*	Azulejo común	ND	Adult	Negative	Negative	Negative	Negative
7		*Zimmerius chrysops*	Atrapamoscas caridorado	ND	ND	Negative	Negative	Negative	Negative
8		*Zonotrichia capensis*	Gorrión de montaña	ND	Juvenile	Positive	Positive	Negative	Negative
9		*Tangara gyrola*	Tángara cabecirufa	ND	ND	Negative	Negative	Negative	Negative
10		*Zimmerius chrysops*	Atrapamoscas caridorado	ND	ND	Negative	Negative	Negative	Negative
11		*Zonotrichia capensis*	Gorrión de montaña	Male	ND	Positive	Positive	Negative	Negative
12	1,760	*Stilpnia vitriolina*	Tángara rastrojera	ND	ND	Positive	Positive	Negative	Negative
13		*Melanerpes rubricapillus*	Carpintero habado	Male	Adult	Negative	Negative	Negative	Negative
14		*Tangara gyrola*	Tángara cabecirufa	ND	Adult	Negative	Negative	Negative	Negative
15		*Tangara arthus*	Tángara dorada	ND	Adult	Positive	Positive	Negative	Negative
16		*Tangara gyrola*	Tángara cabecirufa	ND	ND	Positive	Positive	Negative	Negative

17		*Stilpnia vitriolina*	Tángara rastrojera	ND	ND	Positive	Positive	Negative	Negative
18		*Stilpnia vitriolina*	Tángara rastrojera	ND	Juvenile	Negative	Negative	Negative	Negative
19		*Tangara arthus*	Tángara dorada	ND	ND	Positive	Positive	Negative	Negative
20		*Tangara gyrola*	Tángara cabecirufa	ND	Juvenile	Positive	Positive	Negative	Negative
21		*Eubucco bourcierii*	Torito	Female	Adult	Negative	Negative	Negative	Negative
22		*Eubucco bourcierii*	Torito	ND	Juvenile	Negative	Negative	Negative	Negative
23		*Tangara gyrola*	Tángara cabecirufa	Male	Adult	Negative	Negative	Negative	Negative
24	1,771	*Streptopelia decaocto*	Paloma turca	ND	ND	Negative	Negative	Negative	Negative
25		*Streptopelia decaocto*	Paloma turca	ND	ND	Negative	Negative	Negative	Negative
26	1,772	*Zonotrichia capensis*	Gorrión de montaña	ND	ND	Positive	Positive	Negative	Negative
27		*Thraupis episcopus*	Azulejo común	ND	Immature	Positive	Positive	Negative	Positive
28		*Thraupis episcopus*	Azulejo común	ND	Immature	Negative	Negative	Negative	Negative
29		*Atlapetes albinucha*	Saltón de nuca blanca	ND	ND	Positive	Positive	Negative	Negative
30		*Chlorophanes spiza*	Mielero verde	Male	Immature	Negative	Negative	Negative	Negative
31		*Zonotrichia capensis*	Gorrión de montaña	ND	Juvenile	Negative	Negative	Negative	Negative
32		*Momotus aequatorialis*	Barranquero	ND	Adult	Negative	Negative	Negative	Negative
33		*Molothris bonariensis*	Chamón	Female	Adult	Negative	Negative	Negative	Negative
34		*Zonotrichia capensis*	Gorrión de montaña	ND	ND	Negative	Negative	Negative	Negative
35		*Ramphocelus dimidiatis*	Toche pico de plata	Male	Juvenile	Negative	Negative	Negative	Negative
36		*Tangara gyrola*	Tángara cabecirufa	Male	Adult	Negative	Negative	Negative	Negative
37	2,034	*Tangara arthus*	Tángara dorada	ND	ND	Positive	Positive	Negative	Negative
38		*Elaenia flavogaster*	Elenia copetona	ND	ND	Negative	Negative	Negative	Negative
39		*Stilpnia vitriolina*	Tángara rastrojera	ND	ND	Negative	Negative	Negative	Negative
40		*Thraupis episcopus*	Azulejo común	ND	Adult	Positive	Negative	Negative	Positive
41		*Euphonia laniirostris*	Eufonia gorgiamarilla	Female	ND	Negative	Negative	Negative	Negative

42		*Stilpnia vitriolina*	Tángara rastrojera	ND	ND	Positive	Positive	Positive	Positive
43		*Stilpnia vitriolina*	Tángara rastrojera	ND	ND	Positive	Positive	Positive	Negative
44	2,053	*Elaenia frantzii*	Elenia montañera	ND	Adult	Negative	Negative	Negative	Negative
45		*Elaenia frantzii*	Elenia montañera	ND	Adult	Negative	Negative	Negative	Negative
46		*Diglossa sittoides*	Picaflor canela	Male	Adult	Negative	Negative	Negative	Negative

ND: Not determined.

Discussion

The objective of this study was to conduct an initial survey of the presence of avian Haemosporida parasites infecting birds, in an important area for endemic birds in Colombia. Through morphological identification by microscopy, we determined infection prevalence and explored the characteristics of birds associated with infection. This region is notable for its diverse biomes, but in addition to habitat-related threats, local bird populations may also be vulnerable to infectious diseases, which remain insufficiently investigated but could worsen the impacts on these populations.

We found a high infection prevalence of Haemosporida parasites (34.8%) using morphological identification conducted by microscopic examination of blood of endemic birds (i.e., *Z. capensis, T. episcopus, A. albinucha, T. arthus, S. vitriolina,* and *T. gyrola*). These findings exceed what has been previously reported by González *et al*. (2014; 2015) by 16 and 10%, respectively. The overall prevalence of *Plasmodium* infection (32.6%) also exceeded that in previous reports by González *et al.* (2014; 2015), which were 3 and 4%, respectively. For *Haemoproteus* sp. (6.5%), it falls within the ranges reported previously (González *et al.*, 2014; 2015; Mantilla *et al.*, 2016), and for *Leucocytozoon* (4.3%), it is in line with what was reported by González *et al.* (2014) but much lower than the 56.4% reported by Lotta *et al.* (2016). The differences in the results of bird studies may be due to seasonal variations that affect the composition and health of the populations studied. Factors such as migration patterns, breeding season, food availability, climate, and environmental conditions influence the prevalence of diseases and parasites. Additionally, the age structure of the birds and the stress during certain periods, such as migration or breeding, can affect their immunity, leading to differences in findings between studies conducted in different seasons.

These parasites not only cause reproductive failure in birds but also increase the possibility of coinfections with other infectious agents that take advantage of periods of immunosuppression, aggravating clinical conditions and, therefore reducing the survival chances of the hosts (Levin and Parker, 2012; Chan *et al.*, 2013). In addition, avian

Haemosporida parasites could affect the distribution of host species if the pathogens decrease the fitness of the host (Muriel, 2020). This effect could be more drastic in endemic species with restricted distributions that cannot disperse to disease-free areas. Furthermore, anthropogenic intervention facilitates the rapid spread of infectious diseases, leading to significant environmental catastrophes due to disruptions in species dynamics in most of the cases (Scheele *et al.*, 2019; Woodroffe and Sillero-Zubiri, 2020; Cheng *et al.*, 2021). This directly influences their rapid extinction (Dirzo *et al.*, 2014), with infectious diseases considered one of the five fundamental drivers of the phenomenon worldwide (Smith *et al.*, 2006). Therefore, it is necessary to understand the dynamics of these hemoparasite infections in wild birds, which will contribute scientifically to safeguarding animal health, ecosystem health, and human health to ensure the conservation of wildlife.

Several studies have been conducted worldwide to determine the frequency of Haemosporida infections as well as to explain their dynamic and to better understand how avian populations infected with Haemosporida parasites are affected. However, there is still a lack of comprehensive knowledge about the transmission vectors of Haemosporida species, as well as the varied effects of parasites of the same lineage or species on host species (Santiago-Alarcón *et al*., 2012).

Avian Haemosporida have been extensively studied in various regions of the world (Emmenegger *et al.*, 2023; Harl *et al.*, 2024; Minichová *et al.*, 2024). Research has shown that parasites, such as *Plasmodium* and *Haemoproteus*, can infect a wide range of bird species, with varying prevalences and pathogenic effects (Stager *et al.*, 2021; González-Olvera *et al.*, 2022; Meister *et al.*, 2022). These species have been identified in various avian hosts across different regions, indicating a broad distribution and host range for avian Haemosporida species. The prevalence of these parasites varies among different bird groups, with some lineages showing specific host preferences, while others infect a wider range of avian species.

The population levels of birds in Colombia are highly important in terms of their ecosystems. The IUCN Red List of Threatened Species reports 92 bird species as vulnerable (VU), endangered (EN), and critically endangered (CR), making epidemiological surveillance of diseases that may affect these species, such as Haemosporida parasites, necessary (IUCN, 2023). The presence of vectors capable of transmitting hemoparasites in Colombia, a country in the neotropics, is a reality. This calls for studying the dynamics of these parasites in resident, endemic, and migratory wild birds within the country.

Advances have been reported in the morphological characterization and understanding of the relationships between some vectors and parasite lineages in Colombia. The presence of various vectors and hemoparasites, such as *Plasmodium, Haemoproteus*, and *Leucocytozoon* species, has been reported in the country (González *et al.*, 2014; 2015; Lotta *et al.*, 2016; Mantilla *et al.,* 2016; Lotta *et al.,* 2019; Tamayo-Quintero *et al.,* 2023), posing risks to wild populations and affecting the ecosystem and public health. In addition, ecological variables influencing the prevalence of these pathogens, including elevation, nest height, participation in mixed-species flocks, and host density and diversity, have been identified (González *et al.*, 2014; 2015;Gil-Vargas and Sedano-Cruz, 2019; Tamayo-Quintero *et al.*, 2023).

However, there are still significant scientific gaps regarding the distribution of these pathogens and their biological interactions in different regions of Colombia. Antioquia Province, for example, has a wealth of bird species and vectors that allow for easier observation of the ecology of these parasites, which could contribute to expanding knowledge about those that parasitize wild birds (Pérez-Rodríguez *et al.,* 2014).

Our findings strongly indicate that there are probably more Haemosporida species present in this region than we initially identified. Given the importance of conserving the area and its unique species, it's crucial to further investigate the ecology of these parasites and their relationships with the hosts we studied.

While the sample size of this study is insufficient for statistical comparisons, our results carry significant implications for conserving endemic species in the municipality under study. They also prompt consideration of whether this trend extends to other endemic bird species in the area, as well as to different zones and altitudes within the broader region.

As limitations to our study, in Figure 2, rounded *Haemoproteus* gametocytes are observed, suggesting prolonged exposure of the blood to air, which led to the preparation of mature gametocytes for exflagellation. This process induces morphological changes that could hinder future identification of the parasite at the species level. Blood films must be prepared within seconds after withdrawal from the birds. Otherwise, while the parasite genera can typically still be identified (as was done in this study), species-level identification becomes difficult. This issue arose due to the field conditions during sampling, and as a result, valuable material for taxonomic parasite studies could not be fully utilized.

There's an urgent necessity to evaluate whether Haemosporida parasites poses a threat to the conservation of local bird species. This requires thorough sampling across a wider range of altitudes, encompassing various habitat types and periods as well as genetic analyses on lineage levels. Estimating the prevalence of the parasites in this critical area for endemic birds is paramount for gaining insights into the evolutionary and ecological dynamics of the disease in regions with a high diversity of hosts and parasites. Once screening for Haemosporida parasites becomes routine in wild bird populations, subsequent research should focus on identifying the factors influencing infection and transmission in the area, as well as assessing the direct and indirect effects of Haemosporida infection on different aspects of the host.

Conclusions

In this study, 46 wild birds from 20 species were captured, with an overall parasite infection prevalence of 34.8%. Among them, 32.6% tested positive for *Plasmodium* sp., 6.5% for *Haemoproteus* sp., and 4.3% for *Leucocytozoon sp*., with 6.5% coinfected by multiple parasites. An association between *Plasmodium* infection and age group was observed. This research expands the understanding of hemoparasites in wild birds in Colombia, highlighting the presence of avian hemoparasites. Further studies on molecular identification, pathogenicity, and population impact are needed.

Declarations

Conflicts of interest

The authors declare no conflicts of interest regarding the work presented in this report.

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Author contributions

ACF and NMCV had the idea for the article and led the study conception and design. The samples were collected by ACF and AOAP. The morphological analysis of the samples was performed by AOAP and IJRC. The literature search and data analysis, as well as the critical revision of the manuscript, were performed by all the authors. The first draft of the manuscript was written by AOAP and NMCV, and all the authors commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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