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Research Article

Avian malaria in wild birds within a tropical dry forest area in Guatemala

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Abstract

Urbanization is widely recognized as a driver of increased pathogen transmission, primarily through landscape modification and heightened contact rates between hosts and vectors, which can elevate parasite prevalence and virulence. In this survey, we investigated the prevalence and parasitemia rates of avian malaria in urban and rural areas within a tropical dry forest region of eastern Guatemala—an area undergoing substantial transformation from natural landscapes to urbanized environments. Using a combination of microscopy and PCR-based techniques, we diagnosed and quantified malaria parasites in 181 blood samples collected from 33 wild bird species. Our findings revealed a moderate overall prevalence of avian malaria (38%), with *Plasmodium* spp. being the most prevalent, followed by *Haemoproteus* spp. Notably, no *Leucocytozoon* infections were detected in the sampled populations.

Key words: Avian malaria incidence, *Haemoproteus*, *Plasmodium*, urbanization, vector-borne diseases

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Introduction

Urbanization has been identified as a key factor in pathogen transmission dynamics. Human-induced landscape modifications in urban environments can enhance vector distribution and increase host contact rates, thereby facilitating disease transmission (Bradley and Altizer 2007; Ferraguti et al. 2016). Moreover, urbanization is often associated with higher parasite prevalence and virulence, as well as adverse effects on bird health, including compromised body condition due to exposure to pollutants and lower-quality food sources (Bichet et al. 2013; Bichet et al. 2014).

Research on the relationship between urbanization and avian malaria, a widespread vector-borne disease caused by hemosporidian parasites, has produced varied findings. Most studies report higher infection rates in urban bird populations (Evans et al. 2009; Caizergues et al. 2024; Díaz 2024; Ferraguti et al. 2023; Kimani 2024; Neumann et al. 2024; Riccardi et al. 2024), while others find no significant differences between habitat types (Fokidis

et al. 2008; Jiménez-Peñuela et al. 2021; Figuerola et al. 2024). These differences likely reflect the influence of local environmental conditions that shape malaria transmission dynamics, including factors such as vegetation cover, land use, and especially water availability, which has been shown to be a strong predictor of hemosporidian infection in wild birds (Lachish et al. 2011; Krama et al. 2015). Although rural or more natural areas are generally assumed to experience lower transmission of malaria due to reduced human impact, agricultural activities and habitat disturbance can still influence infection risk (Patz et al. 2000; Fourcade et al. 2014). Given this variability, it is important to better understand how infection patterns manifest in degraded or transitional landscapes, which are increasingly common and may not fit neatly into rural–urban categories.

Understanding these transmission patterns is particularly crucial in regions where native bird species are already facing multiple threats. Habitat loss, climate change, and the introduction of novel pathogens can compound the effects of avian malaria, making disease outbreaks even more detrimental. For instance, in New Zealand, avian malaria has been implicated in the decline of native bird populations, with studies linking higher parasitemia levels to increased mortality risk (Niebuhr et al. 2016). Similarly, in Hawaii, rising temperatures and shifting precipitation patterns have contributed to an increase in avian malaria prevalence, further endangering endemic bird species (Atkinson 2008).

While much of the research on avian malaria has concentrated on mountain ecosystems, dry forest regions undergoing rapid transformation also merit significant attention. In Central America, extensive land-use changes over recent decades—driven by large-scale agricultural expansion, cattle ranching, infrastructure development, and illegal deforestation—have led to widespread degradation and loss of dry forests. These processes have accelerated urbanization across the region, potentially altering vector distributions and host–pathogen interactions. Although vector-borne diseases are prevalent in Central America, the effects of urbanization on their transmission dynamics remain poorly understood (Ortiz et al. 2022). Avian malaria research in the region is similarly sparse, with few studies addressing disease prevalence in wild bird populations or exploring the influence of urbanization on infection patterns. Expanding research in this area is essential for identifying spatial and temporal variations in disease epidemiology and understanding the broader implications of habitat transformation (Meentemeyer et al. 2012).

In this survey, we used both microscopy and PCR methods to examine the prevalence of avian hemosporidian parasites in wild birds from urban and rural areas within a tropical dry forest region in eastern Guatemala. While several studies have investigated the incidence of blood parasites in domestic birds across various localities in Guatemala via microscopic examination, this study is the first to apply molecular methods to the detection of hemosporidian parasites in wild birds in the country. We anticipate variation in the prevalence of hemosporidian genera based on habitat type, as different genera can exhibit distinct responses to environmental changes and conditions.

Methods

Study site

The study was carried out in the Motagua Valley region, located in eastern Guatemala within the department of Zacapa, at elevations ranging from 300–900 m above sea level (Fig. 1).

The Motagua Valley region is characterized by a tropical subhumid climate, with an average annual temperature of 26.9 °C and an average annual precipitation of 815 mm. It experiences a pronounced dry season, typically lasting up to 5 months from November to May. The landscape comprises a mosaic of tropical dry forest patches with varying levels of anthropogenic disturbance, surrounded by diverse human settlements and anthropogenic land use. The land use encompasses a variety of activities, primarily centered around crop cultivation (such as melon, watermelon, lemon, tobacco, mango, and okra), pasture lands, grazing areas, and urban development. For our study, we assessed 12 distinct sites, nine of which were situated in rural environments and six in urban areas (Appendix 1 provide details on the locations and names of the sampling sites).

Bird capture and blood sampling

Wild birds were captured via six 12-meter-long mist nets over a two-day period from 6:30 am to 11:30 am, between March and October 2021. Backyard birds were directly captured at their breeding sites through manual handling. Blood samples were collected from the majority of birds by extracting 20–40 µl from the sub-brachial wing vein using appropriately sized syringes, considering each individual's size and weight (Santiago-Alarcón and Carbó-Ramírez 2015). The volume obtained never exceeded 1% of the bird's body weight (Voss et al. 2010). Samples were preserved in 1 ml of EDTA, kept on ice in the field, and later transferred to −20 °C for long-term storage in the laboratory. For birds weighing less than 3 grams, blood was obtained via a cardiac puncture (Owen 2011). The samples were preserved at −20 °C until genomic DNA extraction. Additionally, each bird underwent sex determination, weight measurement, and comprehensive body morphometrics, including wing length, tail length, beak length, and beak width.

Microscopic examination of blood smears

A small aliquot of the blood collected in the field was used to prepare blood smears immediately following collection. The smears were fixed onsite with pure ethanol once they had dried by wind exposure for 3–4 minutes (Valkiūnas 2005; Santiago-Alarcón and Carbó-Ramírez 2015). Blood smears were then stained with Giemsa diluted in a saline solution at pH 7.2 for 30 minutes and screened at 40× magnification for 30 minutes using an Olympus CX23 light microscope (Olympus, Tokyo, Japan) (Santiago-Alarcón and Carbó-Ramírez 2015). If a sample indicated malaria infection, we estimated the infection rate by calculating the proportion of infected red blood cells relative to the total number of blood cells observed. This was accomplished by counting the number of infected erythrocytes in 10 randomly selected fields on each slide via immersion oil and a 100× objective. Each field typically contained between

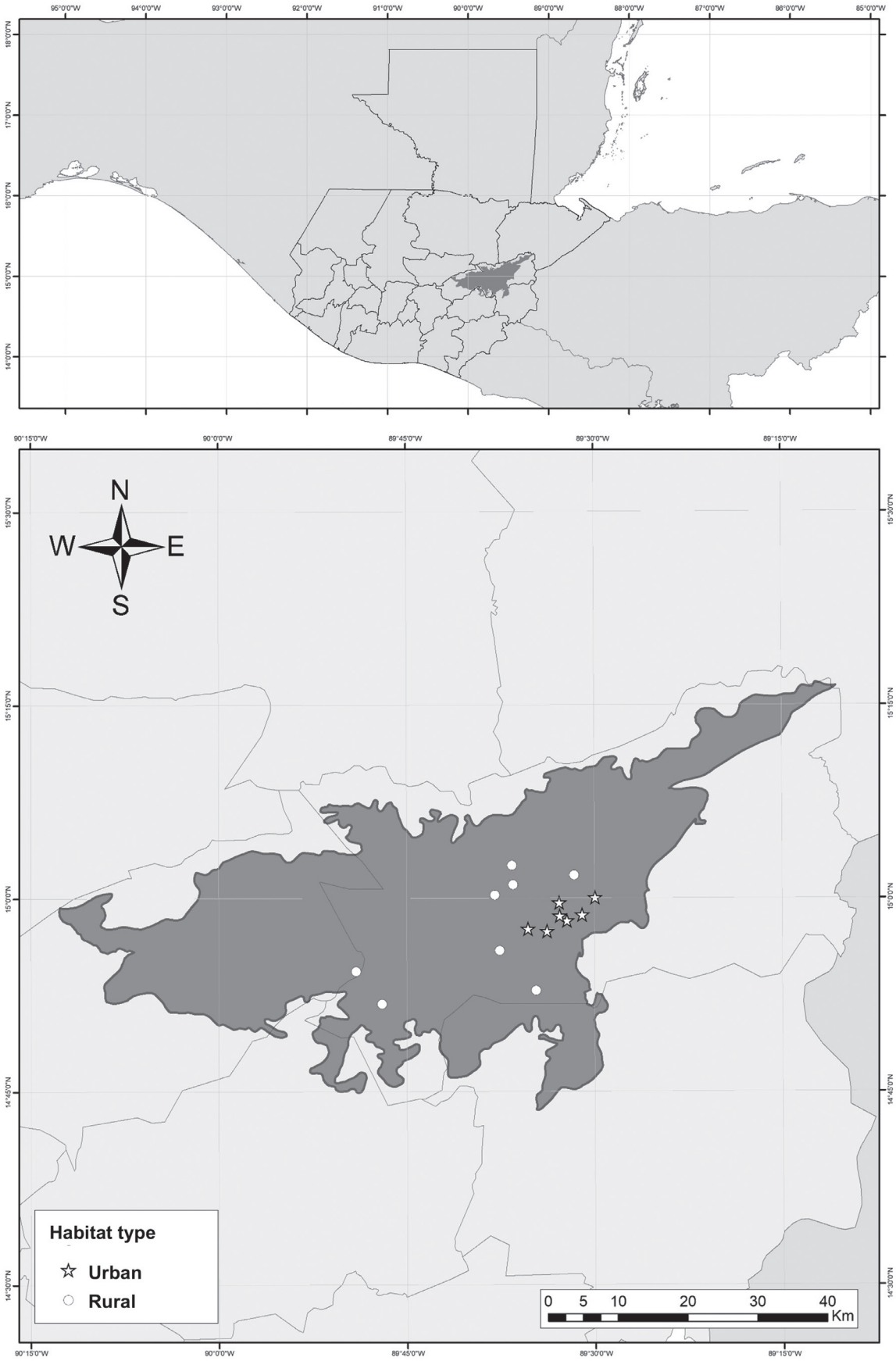

Figure 1. Map of the study site. Left: Location of the semiarid region of the Motagua Valley in Guatemala. Right: Locations of the sampling sites according to habitat type within the semiarid region of the Motagua Valley in Guatemala.

1,000 and 5,000 red blood cells (Santiago-Alarcon and Carbó-Ramirez 2015). The samples were subsequently categorized based on the number of parasites counted relative to the number of red blood cells as follows: low—1 to 10 parasites per 10,000 red blood cells; moderate—11 to 100 parasites per 10,000 red blood cells; moderate to high—1 to 10 parasites per 100 red blood cells; and high—11 or more parasites per 100 red blood cells.

DNA extraction and PCR protocol for avian malaria genus identification

Total DNA for PCR analysis was extracted from each blood sample using the IB47202 Genomic DNA MiniKit (Blood/Cultured Cell) (IBI Scientific, city, country), with 25 µl of blood. We performed multiplex PCR to confirm avian malaria infections (Ciloglu et al. 2019). The primer sets were: PMF [5´-CCT CAC GAG TCG ATC AGG-3´] and PMR [5´-GGAAACCGGCGCTAC-3´] for *Plasmodium*; HMF [5´-ATTGGATGTCAATTACCACAATC-3´] and HMR [5´-GGGAAGTTTATCCAGGAAGTT-3´] for *Haemoproteus*; and LMF [5´-TGGAACAATAATTGSATTATTTACAYT-3´] and LMR [5´-AACATATCATATTCCATCCATTTAGATTA-3´] for *Leucocytozoon*. The PCR reaction had a final concentration of 1× JumpStart™ Taq ReadyMix™, 0.4 µM of each primer, and 50 ng of DNA. Thermocycling conditions were as follows: a) hot-start denaturation at 94 °C for 2 minutes; b) 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 54.5 °C for 30 seconds, and extension at 72 °C for 2 minutes; and c) a final extension step at 72 °C for 5 minutes. Subsequently, 1.5% agarose gels containing GelRed™ (Biotium, Inc., Hayward, CA, USA) were prepared for electrophoresis, performed at 110 V and 90 mA for 40 minutes. Finally, the gels were visualized on a transilluminator to verify the presence of amplification bands (Voytas 1992).

Data analysis

To assess the associations between prevalence and habitat type (rural or urban), we employed Fisher's exact test (Bower 2003). This nonparametric method allowed comparison of the proportion of categories in two independent groups—prevalence (positive and negative) and habitat (urban and rural).

To evaluate the likelihood of avian malaria infection and parasitic load in relation to habitat type, a binomial logistic regression model was used to determine whether malaria prevalence differed between urban and rural sites. All analyses were conducted using R software v.3.0.2 (R Core Team 2024).

Results

Overall prevalence of avian malaria

We examined a total of 150 blood samples collected from 31 distinct bird species in the Motagua Valley region of Guatemala (Table 1). Fifty of the samples came from rural areas and 100 from urban areas. Fifty-six samples (38%) tested positive for avian malaria using both blood smear detection and PCR techniques (Table 1). Specifically, 26 samples (46%) were infected with *Plasmodium*, 14 samples (25%) were infected with *Haemoproteus*, and two samples had mixed infections with parasites from both *Plasmodium* and *Haemoproteus*.

Table 1. Birds sampled for blood parasites of the genera *Plasmodium* and *Haemoproteus*, with the number of parasites corresponding to each malaria genus.

Family and Host Species			Number of individuals infected with parasite genus				
Anatidae	**No. Sampled**	**No. Infected**	**PMR**	**HMF**	**Mixed infection**	**Microscopy results**	**Number of negative samples**
Anas platyrhynchos	4		1				3
Dendrocygna autumnalis	1						1
Cardinalidae							
Passerina caerulea	1		1				
Passerina versicolor	4	2	1			1	2
Pheucticus chrysopeplus	1			1		1	
Pheucticus ludovicianus	1		1				
Columbidae							
Columba livia	1						1
Columbina inca	30	13	5	3		12	17
Columbina passerina	15		7			6	5
Columbina talpacoti	9		2				7
Zenaida asiatica	2						2
Culicidae							
Crotophaga sulcirostris	6						6
Emberizidae							
Peucaea ruficauda	4		1			1	3
Sporophila morelleti	1						1
Icteridae							
Icterus gularis	1				1	1	
Icterus pustulatus	6			4		3	1
Mimidae							
Dumetella carolinensis	1		1				
Mimus gilvus	2			1		1	1
Momotidae							
Eumomota superciliosa	4		1		1		2
Momotus mexicanus	4			1			3
Piscidae							
Melanerpes aurifrons	2					1	1
Polioptilidae							
Polioptila albiloris	8		1	2			6
Strigidae							
Glaucidium brasilianum	2						2
Trochilidae							
Amazilia rutila	2			1		1	1
Troglodítidae							
Campylorhynchus rufinucha	6			1			5
Turdidae							
Turdus grayi	13		2			3	8
Tyrannidae							
Myiarchus nuttingi	1						1
Myiarchus tuberculifer	3		1	1		1	1
Myiozetetes similis	6						6
Pitangus sulphuratus	2						2
Tyrannus melancholicus	7		1			1	6

Dual infections were observed in a Baltimore oriole (*Icterus gularis*) and a turquoise-browed motmot (*Eumomota superciliosa*) (Fig. 2). None of our samples were positive for *Leucocytozoon*.

Parasitemia levels in blood smears

A total of 209 blood smear slides was obtained from the blood samples (for certain individuals, more than two blood smear slides were generated to ensure thorough analysis). After microscopic examination, 33 slides were found to be positive for avian malaria. Notably, approximately 69% of these positive samples presented a moderate level of parasitemia, whereas the other categories collectively constituted less than one-third of the total positive samples (Table 2).

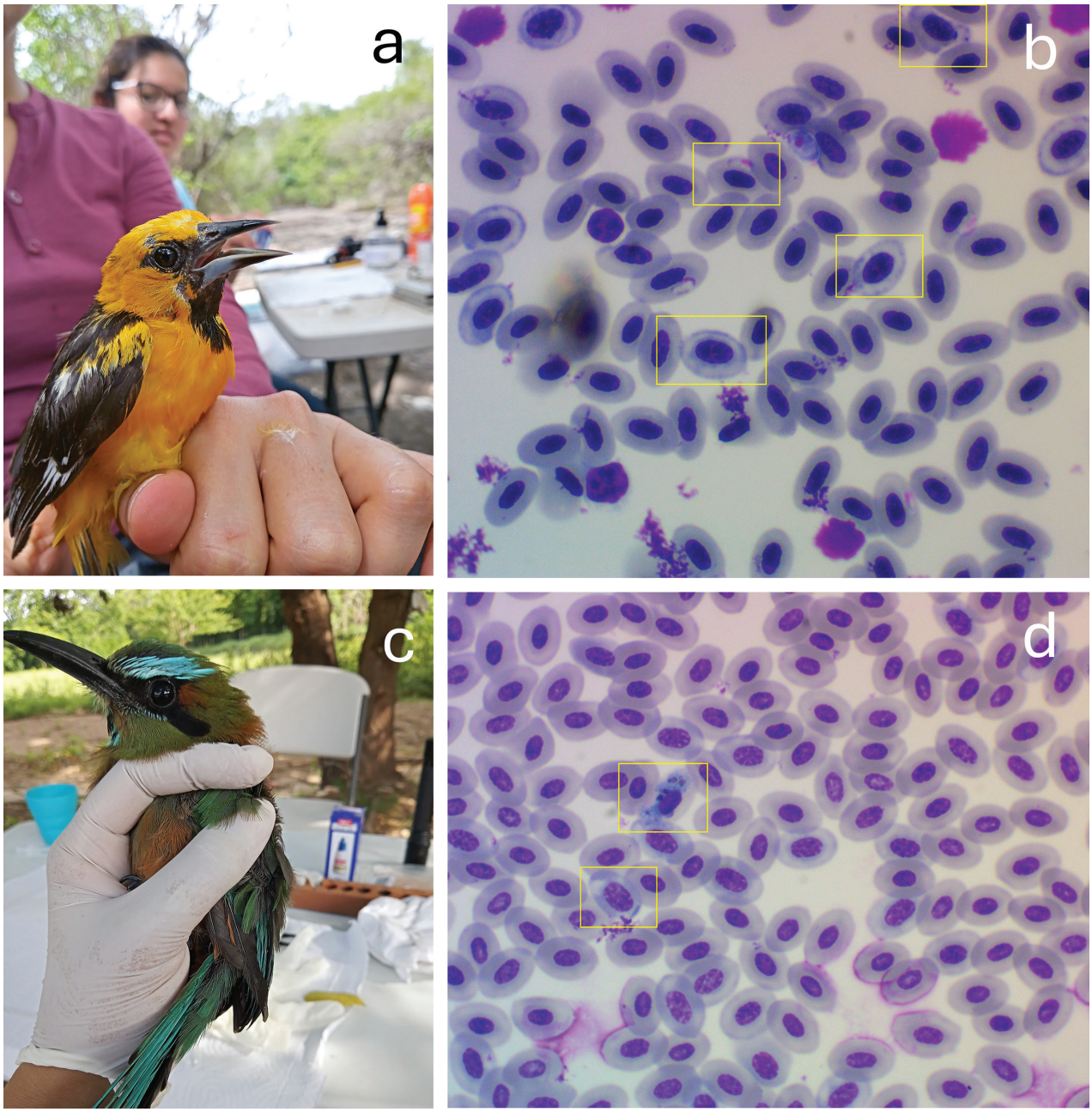

Figure 2. Photographs of avian hosts and blood smears showing infected blood cells with avian malaria parasites. **A**. Baltimore oriole (*Icterus galbula*) in the field; **B**. Blood smear from the same individual, showing infected red blood cells with avian malaria parasites; **C**. Turquoise-browed motmot (*Eumomota superciliosa*) in its natural habitat; **D**. Blood smear from the same individual, displaying infected red blood cells with various stages of malaria infection (arrows).

Table 2. Number of infected erythrocytes in 10 randomly selected fields of slides positive for avian malaria in wild bird species.

Bird specie	Hemosporidian genus	No. of infected erythrocytes in 10 randomly selected fields	Parasitemia level
Amazilia rutila	*H*	0.0008	low
Columbina inca	*H*	0.003	moderate
	P	0.003	moderate
	H	0.018	moderate to high
	H	0.0026	moderate
	Pl	0.0025	moderate
	H	0.0005	low
	H	0.0016	moderate
	H	0.008	moderate
	P	0.0017	moderate
	P	0.012	moderate to high
	P	0.0029	moderate
		0.0021	moderate
Columbina passerina	*H*	0.0012	moderate
	P	0.01	moderate to high
	P	0.0141	moderate to high
	H	0.0035	moderate
	P	0.0024	moderate
	Pl	0.0008	low
Icterus gularis	*P*	0.0053	moderate
Icterus pustulatus	*H*	0.0015	moderate
	H	0.158	high
	H	0.0046	moderate
Melanerpes aurifrons	*P*	0.065	moderate to high
Mimus gilvus	*H*	0.006	moderate
Myiarchus tuberculifer	*P*	0.0044	moderate
Passerina versicolor	*P*	0.0039	moderate
Peucaea ruficauda	*P*	0.0091	moderate
Pheucticus chrysopeplus	*H*	0.016	moderate to high
Turdus grayi	*H*	0.002	moderate
	P	0.017	moderate to high
	H	0.0057	moderate
Tyrannus melancholicus	*P*	0.005	moderate

H = *Haemoproteus* and P = *Plasmodium*.

The results showed no significant difference in prevalence between habitat types (estimate = 0.042, SE = 0.371, z = 0.114, p = 0.909). The odds ratio for urban sites compared to rural sites was 1.04 (95% CI: 0.51–2.18), indicating a negligible difference in infection probability. The likelihood ratio test confirmed that the addition of site type to the model did not significantly improve model fit (p = 0.909).

We found significant variation in *Plasmodium* prevalence across habitat types (x^2 = 10.704, df = 1, p = 0.00106), with higher prevalence in urban areas, while no significant variation was observed for *Haemoproteus* prevalence (x^2 = 0.067, df = 1, p = 0.796).

Discussion

Our overall prevalence of avian malaria parasites is consistent with previous studies conducted in arid ecosystems of the Neotropical region, where prevalence rates typically range from 30% to 50%. For example, similar prevalence rates have been reported in the southern Chihuahuan Desert (32%; Tinajero et

al. 2019) and in tropical dry forests of Brazil (35%; Lacorte et al. 2013). While our findings are lower than those observed in tropical dry forests closer to the equator—such as 51% in Brazil's Caatinga dry forest (Dutra et al. 2023) and in a population of *Zonotrichia capensis* in the Ecuadorian Andes dry forest (Cadena-Ortiz et al. 2018)—they align with the broader range of reported values in arid and semiarid Neotropical regions. These results reinforce the notion that avian malaria infections are not uncommon in species inhabiting arid environments and highlight the substantial variability in prevalence rates even across similar habitats (Santiago-Alarcón and Marzal 2020).

In tropical regions, *Haemoproteus* spp. typically exhibit higher prevalence than *Plasmodium* spp. (Santiago-Alarcón and Marzal 2020). In this study, we found *Plasmodium* to be more prevalent than *Haemoproteus*. In addition, we found no evidence of *Leucocytozoon* infection, aligning with its low prevalence in the Neotropics (Valkiūnas 2005; Atkinson 2008). This parasite, transmitted by Simuliidae black flies, is more common in the Nearctic (Fecchio et al. 2020) and high-altitude Neotropical areas with suitable vector breeding sites. Its absence in the low-altitude Motagua Valley likely reflects unfavorable conditions for these vectors. Further research across altitudinal gradients is needed to clarify the role of altitude in avian malaria dynamics in eastern Guatemala.

Most infected birds in this study exhibited a moderate level of parasitemia. Blood protozoan parasites such as *Plasmodium* spp. often maintain low to intermediate parasitemia in hosts due to host resistance, which promotes long-term parasite survival within populations (Valkiūnas 2005; Dimitrov et al. 2015). Chronic (mild) parasitemia is common in wild birds infected with *Plasmodium* spp., as observed here. Certain bird species, including members of the Columbidae family and some Passeriformes, are considered chronic hosts of hemosporidian parasites, facilitating their persistence in populations. However, susceptibility to infection can vary depending on host-specific conditions (Dadam et al. 2019; Dimitrov et al. 2015).

We observed no significant differences in overall malaria prevalence between rural and urban sites, contrary to our initial hypothesis. This finding is consistent with studies indicating that, while infection risk may decrease in urban settings and increase in rural areas, the observed differences are not always statistically significant. For example, Eurasian blackbirds (*Turdus merula*) in European cities had lower infection rates than rural populations, except in cities with large water bodies that provided ideal vector breeding grounds (Evans et al. 2009). Similarly, in our study, some rural sites near water bodies—such as streams, rivers, or thermal springs (e.g., Emerio Portillo Farms, Carlos Vargas Farm, Niño Dormido, Heloderma Parks, and Sinaneca Village)—may have created localized conditions favorable for transmission. However, these variations were not sufficient to produce an overall difference in prevalence between habitat types.

In relation to parasitemia levels, our data did not show significant differences between urban and rural habitats. Most individuals examined exhibited low to moderate parasitemia levels, regardless of habitat type. These findings point to two potential scenarios: either the avian hosts in the area are in the early stages of infection, or the parasite is causing chronic infections in these hosts (Lacorte et al. 2013). While no statistically significant differences based on habitat type were observed, it is worth noting that within the urban sector, certain individuals presented moderate to high levels of parasitemia, indicating

active disease circulation. The presence of individuals within a population with high parasitemia can enhance parasite transmission to others, as elevated parasitemia increases host infectivity to vectors such as mosquitoes, contributing to host competence and facilitating the spread of avian malaria under suitable environmental conditions (Pigeault et al. 2015).

In our survey, the number of infections detected via PCR differed from those identified through microscopic analysis, consistent with previous research comparing these two methods (Moens and Pérez-Tris 2016; Pacheco et al. 2018). These discrepancies primarily stem from two key factors. First, PCR can detect infections even at extremely low parasite loads—fewer than one parasite per million host cells (Hellgren et al. 2004)—a threshold unlikely to be captured in standard microscopic examinations, which typically analyze 10,000–100,000 cells (Atkinson 2008). Second, some PCR-detected infections may be controlled by the host and never progress to stages circulating in the bloodstream (Valkiūnas et al. 2013). These findings underscore the need for further research to evaluate the efficacy and limitations of both detection methods. Additionally, low parasitemia levels observed in mist-netted birds, as in this study, may be influenced by a capture bias inherent to this method (Mukhin et al. 2016). Studies have shown that high parasitemia significantly impairs bird mobility, reducing the likelihood of infected individuals being captured (Mukhin et al. 2016). These findings suggest that, in natural conditions, avian populations may experience more severe impacts from malaria than observed in mist-net surveys, as heavily infected individuals are less likely to survive or be active and captured.

Conclusion

Our study provides valuable data on the prevalence and parasitemia levels of avian malaria in a tropical dry forest region in eastern Guatemala. In contrast to our initial hypothesis, we found no significant differences in malaria incidence or parasitemia levels between urban and rural bird populations. These results suggest that, while habitat type may influence other aspects of avian malaria dynamics, such as parasite genus distribution, it does not necessarily correlate with overall prevalence or parasitemia intensity. Our findings align with those from similar studies conducted in arid and semiarid Neotropical regions, highlighting the variability and complexity of avian malaria epidemiology in these environments. In addition, our study underscores the limitations and discrepancies between diagnostic methods, with PCR detecting more infections than microscopic examinations. This finding emphasizes the need for continued research to refine and compare detection techniques, which is crucial for accurately assessing the impact of avian malaria on bird populations. Future longitudinal studies across diverse habitats will be essential to fully comprehend the temporal and spatial dynamics of avian malaria, especially in regions experiencing rapid environmental changes due to human activities.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Conceptualization: MBC. Data curation: EDLR, LACA. Formal analysis: MABI.

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Data availability

Species occurrence data collected during this study are available at GBIF, the Global Biodiversity Information Facility, at https://doi.org/10.15468/n4sd8w, accessed via GBIF.org on 1 August 2025.

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Appendix 1

Table A1. Study site locations.

Location	Municipality and departmente	Coordinates N, W	Habitat type
Parque Regional Municipal Niño Dormido	Cabañas, Zacapa	14.90653794, -89.81526	rural
Reserva Natural Privada Heloderma	Cabañas, Zacapa	14.862972, -89.78247	rural
El Guayabal	Estanzuela, Zacapa	15.004, -89.63222	rural
Finca Emerio Portillo	Estanzuela, Zacapa	15.017111, -89.60808	rural
Instituto de Ciencia y Tecnología Agrícolas	Estanzuela, Zacapa	14.960444, -89.58814	Urban
Parque Regional Municipal El Castillo	Rio Hondo, Zacapa	15.042361, -89.6095	rural
Sinaneca	San Jorge, Zacapa	14.880611, -89.57786	rural
El Terrero	Zacapa, Zacapa	15.000722, -89.49903	urban
Finca Carlos Vargas	Zacapa, Zacapa	14.99425, -89.54661	urban
Finca Bryan López	Zacapa, Zacapa	14.978306, -89.51611	urban
Finca Jairo Trabanino	San Juan, Zacapa	14.93225, -89.62606	rural
San Pablo	San Pablo, Zacapa	15.0295, -89.52667	rural