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## Prevalence and blood parasitaemia of Eurasian Collared Doves (*Streptopelia decaocto*) and Mourning Dove (*Zenaida macroura*) in Durango, Mexico

Prevalencia y parasitaemia sanguínea de palomas turcas (*Streptopelia decaocto*) y huilotas (*Zenaida macroura*) en Durango, México

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### Abstract

Blood parasitism in Mexican birds is an impoverished-studied phenomenon, and its presence in many species of birds is unknown. In this study, the prevalence and parasitaemia of hemosporidia were compared in the breeding (wet) and non-breeding (dry) seasons in the Eurasian collared doves (*Streptopelia decaocto*) and Mourning ones (*Zenaida macroura*) in northern Mexico. The blood of 40 birds of each species collected between 2013 and 2014, was analyzed. The diagnosis of hemoparasites was made by microscopy and Polymerase Chain Reaction (PCR) techniques. The prevalence of hemoparasites was 87.5% (CI 95% = 78.3–93.3). The mean parasitaemia was 7.03 (CI 95% = 5.68–9.04) hemoparasites per 10,000 infected erythrocytes. The prevalence and parasitaemia were higher for *Haemoproteus* sp. than for *Plasmodium* sp and microfilariae. The prevalence rates did not vary between bird species, nor between times of the year. However, seasonality seems to be an important factor in parasitaemia. The species that obtained the highest rates of parasitaemia was *Z. macroura*. More studies are needed to understand the mechanisms that associate parasitaemia in this species with respect to other columbiform species.

**Keywords:** *Streptopelia decaocto*, *Zenaida macroura*, seasonal variation, *Haemoproteus*, *Plasmodium*.

### Resumen

El parasitismo sanguíneo en aves de México es un fenómeno poco estudiado y su presencia en muchas especies de aves es desconocida. En este estudio, se compararon la prevalencia y parasitaemia de hemosporidios en la época reproductiva (húmeda) y no reproductiva (seca) de las palomas turcas (*Streptopelia decaocto*) y huilotas (*Zenaida macroura*) del norte de México. Se analizó la sangre de 40 aves de cada especie, colectadas entre 2013 y 2014. El diagnóstico de hemoparásitos se realizó mediante técnicas de microscopía y reacción en cadena de la polimerasa (PCR). La prevalencia de hemoparásitos fue de 87.5 % (IC 95% = 78.3–93.3). La parasitaemia promedio fue de 7.03 (IC 95 % = 5.68–9.04) hemoparásitos por cada 10,000 eritrocitos infectados. Las tasas de prevalencia y parasitaemia fueron mayores para *Haemoproteus* sp., que en *Plasmodium* sp y microfilarias. Las tasas de prevalencia no variaron entre especies de aves, ni entre épocas del año. No obstante, la estacionalidad parece ser un factor importante en la parasitaemia. La especie que obtuvo mayores tasas de parasitaemia fue *Z. macroura*. Se necesitan más estudios para comprender los mecanismos que asocian la parasitaemia de esta especie con respecto a otras especies de columbiformes.

**Palabras clave:** *Streptopelia decaocto*, *Zenaida macroura*, variación estacional, *Haemoproteus*, *Plasmodium*.



## INTRODUCTION

Exotic species represent a threat to the structure, function and integrity of ecosystems ([Ferreira et al., 2021](#)), contribute to the extinction of some species ([Rocha et al., 2021](#)) and introduce infectious agents to the colonized environment ([Hernandez-Colina et al., 2021](#)). The Eurasian collared dove (STRDEC, *Streptopelia decaocto*) is an invasive species with a wide distribution in North America ([eBird, 2021](#)). It is a relatively large bird ([Salazar-Borunda et al., 2019](#)), which competes for food and nesting sites with local avifauna ([El-Mansi et al., 2021; Koenig, 2020](#)) or can transmit diseases to the colonized environment ([Stilmmelmayr et al., 2012](#)). In Mexico, Mourning Dove *huilota* (ZENMAC, *Zenaida macroura*) shares habitat with *S. decaocto* ([Otis et al., 2020](#)) and represents a model species to study the effects of the exotic species on Mexico's avifauna.

Although the introduction of exotic diseases is a complex phenomenon and multiple etiological agents may be involved ([Hawkins, 2021; Martínez-Pérez et al., 2021](#)), hemoparasites have been widely distributed among birds ([White et al., 1978; Starkloff et al., 2021](#)). This group of microorganisms is transmitted by dipteran insects such as mosquitoes, hypobossids and simulids and include the genera *Haemoproteus*, *Plasmodium*, *Leucocytozoon*, *Falissia*, *Garnia* ([Valkiūnas & Iezhova, 2018](#)), *Trypanosoma* ([Ham-Dueñas et al., 2017](#)) and microfilariae ([Noden et al., 2021](#)).

When infecting birds, hemoparasites can cause acute or chronic clinical signs. In the acute presentation, the host develops a high parasitaemia associated with systemic phenomena generated by exoerythrocytic and intraerythrocytic forms (microgametocytes, macrogametocytes and meronts). The chronic phase instead, occurs days or weeks after infection, when infected birds experience low parasitaemia and mild clinical impacts that can last for years with seasonal relapses ([Valkiūnas & Iezhova, 2017](#)). Although common in bird populations ([Palinauskas et al., 2011](#)) they are sometimes fatal ([Cardona et al., 2002; Yoshimoto et al., 2021](#)), especially when introduced ([Warner, 1968](#)).

In terms of prevalence, hemoparasites vary between ecological regions ([Loiseau et al., 2012](#)), seasons of the year ([DeBrock et al., 2021](#)) or depending on vector response to climatic fluctuations ([Wood et al., 2007](#)). Knowledge of the hemoparasitic prevalence of an exotic and a native species will enrich the biological knowledge of this ecological interaction. Therefore, the objective of this study was to determine and compare the hemoparasitic prevalence between collared doves and mourning doves in Durango municipality, Mexico, during two seasons of the year.



## MATERIAL AND METHODS

The study area corresponds to the localities from "José Refugio Salcido" (23.97 N, -104.51 W), "Praxedis Guerrero Nuevo" (23.94 N; -104.56 W) and "La Purísima" (23.96 N; -104.57 W) from Durango municipality, Mexico. Blood samples (500 µl) were extracted from STRDEC (n= 40) and ZENMAC (n= 40), during the breeding (spring-summer, 2014) and non-breeding (autumn-winter, 2013) seasons under the protection of scientific collection permit SGPA/DGVS/12294/13.

### Blood collection

Two blood smears were taken from each bird, which were dried at room temperature (2 min) and fixed with 100% methanol (3 min). Once dried, they were wrapped in paper to avoid direct contact between them, and subsequently stained with a Giemsa solution (pH 7.0-7.2 at 18-20 °C for 1 h; [Santiago-Alarcón & Carbó-Ramírez, 2015](#)). From the collected blood, 100 µl were deposited in a sterile Eppendorf tube with buffer solution (100 mM Tris HCl, pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5 % SDS; [Longmire et al., 1988](#)) to keep it frozen (- 20 °C) until molecular analysis.

### Molecular analysis

The presence and absence of hemoparasites was determined by polymerase chain reaction (PCR) targeting a 479 base pair region of the cytochrome b gene ([Hellgren et al., 2004](#)). DNA extraction was performed following the DNeasy blood & Tissue® protocol ([Quiagen, 2021](#)). PCR was performed with 100 ng of GA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3.0 mM MgCl, 0.4 mM deoxynucleotide triphosphate, 5 µl Q buffer and 0.5 µl Taq. Amplification was performed from the oligonucleotides HaemNFI-HaemNR3 ([Hellgren et al., 2004](#)) and HaemF-HaemR2 ([Bensch et al., 2000](#)). The products of that reaction were deposited inside an electrophoresis chamber (25 mA, 25 min) together with 0.01 µg/µL TrackIt™ and SYBR® for observation in an ultraviolet light photo-documentation chamber (ImageQuant LAS 4000, 6 s exposure).

### Microscopic analysis

To confirm the presence or absence of parasitic structures, blood smears were examined at high dry (40 ×, 30 min) and wet (100 ×, 30 min) magnification for at least 100 fields using a binocular light microscope (Carl Zeiss®, Primo Star model). Parasite identification, limited to genus level, was performed following the criteria of Valkiūnas and Iezhova ([2018](#)). The prevalence of hemoparasites was estimated by the ratio of infected birds to the total number of sampled individuals.

To determine hemoparasite parasitaemia, the observed parasitic elements were counted by counting 10 000 host red blood cells ([Godfrey et al., 1987](#)).

### Statistical analysis

Prevalence, mean parasitaemia and their respective 95% confidence intervals were calculated with Quantitative Parasitology QPweb ([Reiczigel et al., 2020](#)). Estimation of prevalence as for parasitaemia, was based on 5 000 bootstrap replicates, with the



Sterne method for binomial prevalence data (Rózsa *et al.*, 2000; Ham-Dueñas *et al.*, 2017; Reiczigel *et al.*, 2019). Prevalence and parasitaemia indices were compared between parasitic genera (*Haemoproteus* sp. vs. *Plasmodium* sp.). Prevalence was analyzed by Chi-square analysis and parasitaemia with a generalized linear model (GLM), using the negative binomial distribution.

The year season effect and species were assessed by generalized linear mixed models (GLMM, Paterson & Lello, 2003,), using logistic regression for hemosporidia prevalence and negative binomial distribution for parasitaemia . These analyses were implemented using the "MASS" package (Venables & Ripley, 2002) in R version 4.0.5 (R Core Team, 2021).

## RESULTS

Of the 80 pigeons tested for hemoparasites with PCR, 70 were infected (87.5 %, 95% CI=78.3-93.3). Prevalence rates ranged from 80-100 % and the parasite gene was amplified at a higher rate during the non-breeding season (Table 1).

**Table 1. Percentage of birds infected with hemoparasites, diagnosed morphologically and through amplification of the mitochondrial parasitic cytochrome B gene in Durango, Mexico**

Species	Morphological diagnosis	
	Reproductive*	Non-reproductive **
ZENMAC	14 n, 70 % P, 47.5–86.0 IC	19 n, 95 % P, 75.6–99.7 IC
STRDEC	13 n, 65 % P, 42.4–83.3 IC	14 n, 70 % P, 47.5–86.0 IC
Diagnóstico molecular		
Species	Reproductive*	
	17 n, 85 % P, 62.8–95.8 IC	20 n, 100 % P, 83.3–100 IC
ZENMAC	16 n, 80 % P, 57.6–92.9 IC	17 n, 85% P, 62.8–95.8 IC

ZENMAC *Zenaida macroura*, STRDEC *Streptopelia decaocto*, n Number of infected birds, P Prevalence, 95% confidence interval CI, \*Spring-Summer 2014, \*\*Fall-Winter 2013

Although most infections were diagnosed by both methods, microscopic analysis reflected lower prevalences. Most of the positive samples had single hemosporidia infections. However, one bird of each species showed co-infection between *Haemoproteus* sp. and microfilariae.

The prevalence of hemoparasites detected by light microscopy was 75.0 % (60 birds, 95 % CI= 64.4-83.4) and identified on average 7.03 (95 % CI= 5.68-9.04) hemoparasites per 10,000 infected erythrocytes. The percentage prevalence and average parasitaemia for each species, hemoparasite taxa and season are shown in Table 2.

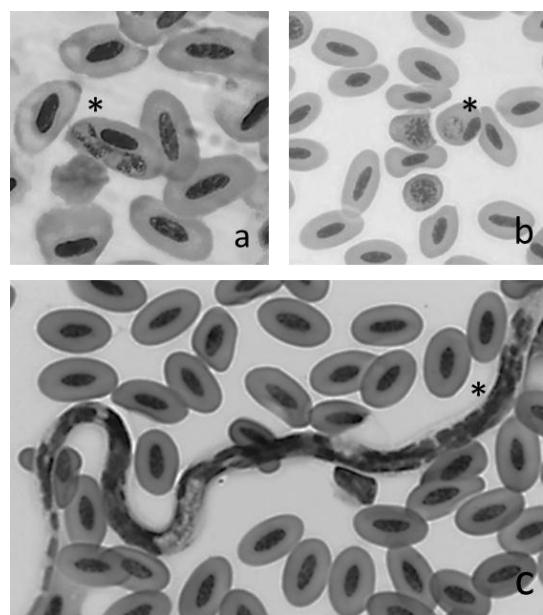
Parasite structures were identified at different evolutionary stages. Macro- and microgametocytes for *Haemoproteus* sp., meronts for *Plasmodium* sp. and larval stages of filariae were detected (Figure 1). *Haemoproteus* sp. infections were higher than *Plasmodium* sp. infections (prevalence:  $X^2 = 1.14$ , df = 1,  $P = 0.02$ , parasitaemia :  $F = 508.80$ ,  $P = 0.001$ ).



**Table 2. Percentage prevalence of hemoparasites and average parasitaemia in *Streptopelia decaocto* and *Zenaida macroura* during the breeding and non-breeding season in Durango, Mexico**

Group	Infected birds	n	Prevalence% (CI 95%)	Parasitaemia <sup>1</sup> average (CI 95%)
STRDEC	27	40	67.5 (51.3–80.2)	4.0 (3.1–5.2)
ZENMAC	33	40	82.5 (67.7–91.6)	9.4 (7.4–12.8)
<i>Haemoproteus</i> sp.	55	80	68.8 (57.5–78.3)	6.3 (5.0–8.17)
<i>Plasmodium</i> sp.	17	80	21.2 (13.6–31.8)	2.6 (1.9–3.4)
Microfilarias	4	80	5.0 (1.7–12.3)	3.0 (1.0–4.7)
Reproductive*	27	40	67.5 (51.3–80.2)	5.15 (3.8–7.4)
Non-reproductive **	33	40	82.5 (67.7–91.6)	8.58 (6.67–11.9)

<sup>1</sup>Number of parasitic elements in 10 000 red blood cells quantified, n Total samples, 95% confidence interval CI, STRDEC collared dove, ZENMAC mourning dove, \*Spring-Summer 2014, \*\*Fall-Winter 2013



**Figura 1. Photomicrographs of blood smears from columbiform birds naturally parasitized by hemoparasites (\*). a. *Haemoproteus* sp., b. *Plasmodium* and c. Microfilariae (c); Giemsa, 100 x**

Generalized linear mixed models revealed that parasitaemia varied between species and time of year ( $F= 337.8$ ,  $P= 0.001$ ). The GLMM fit explaining parasitaemia was moderate ( $R^2 = 0.89$ ). Parasitaemia was higher during the non-breeding season, especially in ZENMAC (Table 2). Finally, the effect of species ( $P= 0.18$ ) and season ( $P= 0.50$ ) on prevalence rates were not significant.



## DISCUSSION

The hypothesis that prevalence rates differ significantly between bird species and between seasons was not supported by the data. This means that the phenomenon of parasitism is common in both bird species.

The prevalence value recorded is higher than some values reported in Mexico ([Reinoso-Pérez et al., 2016](#); [Ham-Dueñas et al., 2017](#); [Villalva-Pasillas et al., 2020](#)). However, these results should not be surprising because the response towards blood parasitism depends on factors associated with seasonality, immunology or host behavior, as reported in similar studies in passerine ([Lee et al., 2006](#); [Dubiec et al., 2016](#)) and columbiform birds ([Schumm et al., 2021](#)).

The influence of such variables on blood parasites is monitored in long-term studies, and generally do not reflect significant differences between prevalences over several years ([Bensch et al., 2007](#); [Dubiec et al., 2016](#)). In this sense, although the patterns observed in this study can be considered reliable, they should be monitored in the same region over time.

On the other hand, the results of this study support the hypothesis that parasitaemia varies between species and seasons. The reasons why the amount of hemoparasites was significantly higher in ZENMAC may be associated with differences in the distribution of both birds or with characteristics of vectors and parasites themselves ([Reinoso-Pérez et al., 2016](#)). Fokis *et al.* (2008), reported a lower incidence of hemoparasites in birds of urban habits and, although both species come to share habitat ([Green et al., 2020](#)), the ZENMAC captured for this study were distributed towards more open areas, with adjacent water bodies, probably with more vectors ([Lega et al., 2017](#); [Lynton-Jenkins et al., 2020](#)) and with higher contagion probability ([Hellard et al., 2016](#)). The influence of season (breeding) on parasitaemia could be considered normal, coinciding with the vector breeding season ([Inumaru et al., 2021](#)) and hormonal events relevant to the resolution of parasitaemia by the avian host ([Deviche & Parris, 2006](#)).

Although regional differences may exist, *Haemoproteus* is the most common hemoparasitic genus in birds, followed by *Plasmodium* and *Leucocytozoon* ([Carlson et al., 2013](#); [Heym et al., 2019](#)). This pattern was observed in this study with the exception of the genus *Leucocytozoon*, whose absence could be associated with the altitudinal and climatic characteristics of the study site that restrict mosquito abundance and parasite development in these vectors ([Borji et al., 2011](#); [Nath et al., 2014](#)). The results show the study site having a sufficient number of vectors capable of transmitting hemoparasites in both bird species ([Valkiūnas & Iezhova, 2018](#); [Inumaru et al., 2021](#)). Works analyzing parasitaemia are limited ([Huang et al., 2020](#)) and associate this parameter with the damage generated by the parasite to the host ([Knowles et al., 2010](#); [Muriel, 2020](#)). To our knowledge, there is no study examining blood parasitaemia in the species sampled in this study. However, the quantified parasitaemia rates were lower than those reported in columbiformes from South Africa ([Nebel et al., 2020](#)), Canary Islands ([Foronda et al., 2004](#)) and India ([Gupta et al., 2011](#)), whose adverse effects on hosts depended on factors such as bird immunity or food availability ([Chagas](#)



*et al.*, 2016). Likewise, it should be considered that under normal circumstances, the *Haemoproteus* and *Plasmodium* genera only cause health problems when the host is cured by stress events, immunosuppression (Valkiūnas & Iezhova, 2017) or when introduced into non-native communities (Yoshimoto *et al.*, 2021), therefore, these phenomena should be ruled out in these species.

In Mexico, studies reporting microfilariae in birds are scarce and their impact on the host is believed to be not very severe (Yanga *et al.*, 2011). Although it was the least observed parasite, the calculated prevalences exceed those reported by similar studies in Mexico (Clark & Swinehart, 1969; Villalva-Pasillas *et al.*, 2020). However, the future development of hemoparasites should be monitored and the possible effects on columbiform communities should be determined, especially in the scenario of global change, since the increase in temperature and anthropogenic changes in land use could provide new opportunities for the transmission of these microorganisms to poultry communities.

It should be noted that in this study were used two analyses (molecular and microscopic) to assess hemoparasite prevalence as accurately as possible. These two approaches led to discrepancies between analyses, determining a lower prevalence in blood smear counts. This event is consistent with previous studies in columbiformes, where the prevalences of microscopic and molecular analysis were different (Dunn *et al.*, 2017; Tavassoli *et al.*, 2018).

Birds whose blood had absence of parasitic structures, but were PCR positive, could be having a slight parasitaemia with few gametocytes, sporozoites or parasite remnants that interrupted their development (Valkiūnas & Iezhova, 2017). The molecular method instead, relied on the detection of the parasite gene, but does not reveal whether the parasites have had or will develop into a successful infection (Chagas *et al.*, 2016; Valkiūnas & Iezhova, 2017).

## CONCLUSION

Blood parasitism was observed in birds of both species, mainly by the genus *Haemoproteus* spp. Parasitaemia was higher in *Z. macroura* during the breeding season and therefore, seasonality should be an important variable to consider in studies involving parasitism in this species. This study contributes to the understanding of the diversity of hemoparasites infecting wild birds of the order Columbiformes in Durango, Mexico. Although we were unable to determine the cause of differences in calculated parasitaemia, this study provides baseline information for monitoring bird populations at the study sites or possible future changes in parasite ranges and diversity.

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Errata Erratum

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