Apparent Absence of Avian Malaria and Malaria-Like Parasites in Northern Blue-Footed Boobies Breeding On Isla Isabel

Federico Roldán-Zurabián
National Autonomous University of Mexico
María José Ruiz-López
Estación Biológica de Doñana
Josué Martínez de la Puente
University of Granada
Jordi Figuerola
Estación Biológica de Doñana
Hugh Drummond
National Autonomous University of Mexico
Sergio Ancona (sergio.ancona@iecologia.unam.mx)
National Autonomous University of Mexico

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Abstract

Haemosporidian parasites are common in birds, but often are not in seabirds. The absence of vectors/genetic resistance to infection have been proposed to explain this pattern. Examination of different host populations is required to confirm the absence of blood parasites in widespread host species, which could be differently exposed to blood parasites across their geographic range. Moreover, screening of blood parasites in many seabirds has been done only by visual inspection of blood smears, which can miss low-intensity infections. Screening of blood parasites of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, combining inspection of blood smears and PCR-based detection methods, revealed that a highly philopatric colony of blue-footed boobies (*Sula nebouxii*) in the Tropical North Pacific is likely free of these parasites. Earlier detection of *Haemoproteus* parasites in frigatebirds cohabiting with boobies in our study site and blue-footed boobies breeding on the Galapagos Islands suggests that absence of blood parasites in this northern booby colony could not be attributable to the absence of vectors or genetic resistance to infection. High host specificity or fine-scale spatial heterogeneity in the abundance of insect vectors could explain our negative results, but these hypotheses remain to be tested. We emphasize the relevance of assessing the occurrence of blood parasites in different populations of widespread host species, such as blue-footed boobies.

Introduction

Plasmodium, *Haemoproteus*, and *Leucocytozoon* blood parasites are widespread parasites causing avian malaria and avian malaria–like diseases [1] that have deleterious impacts on wild birds [2–4]. However, infections by these parasites are not homogeneously distributed among bird taxa, with some groups, including seabirds, usually showing an extremely low prevalence or total absence of parasite infections [5]. Different hypotheses have been proposed to explain these patterns including that absence of parasites is the result of adverse conditions for vectors in seabird habitats [6]. Seabird habitats usually have high salinity, high wind exposure and low vegetation cover, which are expected to impose strong selective pressures on vectors making them infrequent [6,7]. But even in the co-occurrence of potential vectors, blood parasite infections in seabirds are still uncommon [5], suggesting that alternative hypotheses could also explain the observed patterns. Scarcity of infection in seabirds could also be due to good immunological capabilities of the hosts to repel infections, short exposure times to parasitic infections, or lack of suitable host-parasite assemblages [5,8,9].

Blue-footed boobies are socially monogamous seabirds [10] that breed colonially on islands of the Eastern Tropical Pacific Ocean, from Mexico's Gulf of California to northern Peru [11]. Birds nest on the ground in open sites or in areas with moderate vegetation cover [11]. Isla Isabel boobies show lifetime fidelity to their first breeding site in their natal colony [10,12] and may live 20 years or longer [13]. Previous studies on this species revealed an approximately 33–83% prevalence of *Haemoproteus* sp. on the Galapagos islands [14], implying physiological compatibility between birds and blood parasites. These infections were only

detected when using molecular tools; in blood smears only a parasite tentatively identified as *Leucocytozoon* sp. was found.

Here, we combined microscopic inspection of blood smears and molecular screening to assess the prevalence, and eventually the genetic identification, of blood parasites belonging to the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* in adult blue-footed boobies breeding in an insular and highly philopatric colony on Isla Isabel (Mexico). We expect Isla Isabel boobies to be infected by blood parasites for two reasons. First, several species of mosquitoes belonging to the genera *Aedes*, *Anopheles*, *Culex* and the potential vectors of *Haemoproteus* and *Leucocytozoon*, including *Culicoides*, hippoboscids and black flies [15], are widely distributed in Mexico and have been recorded in the study area ([16,17], authors per. obs.). Second, the prevalence of *Haemoproteus iwa* in Isla Isabel frigatebirds, likely vectored by hippoboscid flies [18], has been estimated to be 16% (n = 251 frigatebirds; [19]), reinforcing the idea that there are suitable insect vectors of haemoparasites on the island.

Results

A total of 64 blood smears from 33 male and 31 female blue-footed boobies were visually inspected for the presence of blood parasites. No parasites were detected in blood smears. None of the 64 samples of blue-booted boobies tested molecularly showed evidence of parasite amplification.

Discussion

The inspection of Haemosporidian parasites of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, combining microscopic screening of blood smears and a broadly used PCR-based detection method, suggests that adult male and female blue-footed boobies breeding on Isla Isabel are likely free of these blood parasites. Our results support previous findings by Clark and Swinehart based on blood smears [20] who recorded the absence of these parasites in 19 blue-footed boobies from islands off the North Pacific coast of Mexico (not including Isla Isabel boobies). These negative results reflect the overall scarcity of blood parasites in seabirds, which is often attributed to unsuitable conditions for vectors in seabird habitats [5,21].

In addition to the occurrence of potential vectors in the area, *Haemoproteus* parasites have been previously detected in frigatebirds from Isla Isabel (prevalence ranging from 16 to 54%; [19,22]), thus lack of blood parasites in Isla Isabel boobies probably implies that parasite infections vary among avian host species that coexist on the island. Differences in the prevalence of blood parasites between boobies and frigatebirds could be due to differences in their habitat use or in the abundance and activity of vectors [23,24]. Infected frigatebirds were sampled in the southwestern end of the island, in a shady, vegetated area with large trees located a few ten of meters from an unfinished building where rainwater accumulates, and 150 meters from the only pool of alkaline water on the island [19,25]. This area is protected from the wind and suitable for development and proliferation of some potential vectors [26]. In addition, the

parasites detected in frigatebirds are transmitted by louse flies (Hippoboscidae) that spend most of the time on their vertebrate hosts and present higher host specificity than mosquitoes, *Culicoides* and black flies. Boobies breed mostly where we sampled them in the wind-exposed northeastern end of the island, ~ 1.0–1.5 km from the source of alkaline water pool and the unfinished building where rainwater accumulates. The northeastern area is mainly covered by stunted woody vegetation, since big trees have been recurrently damaged by hurricanes [27,28]. Windy, dry, and hot conditions limit the abundance and activity of potential vectors of blood parasites in marine environments [6,23], and their prevalence in the Isla Isabel booby colony may explain the absence of blood parasites in boobies.

Lack of blood parasites in seabirds has been attributed to evolutionary and ecological factors other than spatial heterogeneity in the occurrence of suitable vectors [5,8], but it is unlikely that these alternative mechanisms explain the results reported here. Earlier screening of blood parasites in boobies from the Galápagos archipelago revealed the infection by a tentatively identified *Leucocytozoon* parasite, and especially, by parasites of *Haemoproteus* genus [14,18]. Thus, it is unlikely that blood parasites are absent from Isla Isabel boobies because they exhibit high immunological capacities, have short exposure times to parasitic infections or present physiological incompatibility with haemoparasites that prevents the latter from completing their life cycles [8,9]. In addition, differences in host numbers [8] may not explain differences in the occurrence of blood parasites between the booby populations of Isla Isabel and the Galápagos. The booby colony of Isla Isabel has up to 1769 breeding pairs in our study area alone, which covers 26, 889 m^2 and contains approximately 65% of all breeding pairs that are established annually on the island [29]. The breeding population of blue-footed boobies in the whole Galápagos archipelago was estimated at 3,200 pairs at the time when high prevalence of blood parasites was detected in Galápagos boobies [14,30]. Thus, host numbers are not different enough between the two populations to explain the apparent lack of blood parasites in Isla Isabel boobies.

In conclusion, we failed to find evidence of blood parasites in one of the largest colonies of blue-footed boobies of the North Pacific coast, after microscopic examination of blood smears and state-of-the-art molecular analysis for detection of avian blood parasites [9,31]. Apparent absence of blood parasites in Isla Isabel boobies indirectly adds to the growing evidence of variation in parasite infections among avian host species that coexist locally [23,32], and highlights the relevance of performing evaluations of the prevalence of blood parasites in different populations of widespread host species [21].

Methods

Study site

Isla Isabel is an 82-ha volcanic island 28 km off the west coast of Mexico, in the Eastern Tropical North Pacific (21º 52´ N, 105º 54´W). The island is mainly covered by deciduous dry forest of *Crataeva tapia* trees, *Euphorbia schlechtendalli* bushes, and an understory dominated by coastal grasslands. The climate is sub-humid tropical with rains in June–November (the hurricane season). In the rainy season,

water levels rise both on the steep rocky slopes and valleys, and water is collected in three endorheic basins in the center and north of the island. There is also a shallow pool of alkaline water of approximately 50 m in diameter in the south of the island that is the result of rainwater runoff and the entry of seawater during storms [27].

Field procedures

In March-April 2019, we hand-captured 64 adult blue-footed boobies (33 males and 31 females) on their nests (sites with a clutch or brood) at night. We recorded the identity of boobies that had a metal ring (since 1989, tens of thousands of fledglings and adults have been banded on Isla Isabel [13]) and sexed by voice (females grunt, males whistle). See Drummond et al. [33] for further details on the field procedures used. We blood sampled all captured boobies only once. Approximately 1.5–2.0 ml of blood were taken from their brachial vein. A drop of blood was used for blood smear preparation and 200 µl were split in two aliquots (100 µl each) and stored in 96% ethanol for molecular screening of blood parasites. The remaining blood was centrifuged and stored for future research. Blood smears were fixed in 96% ethanol and subsequently stained with Giemsa. Manipulation of booby adults took less than 10 min and bleeding stopped before release at the site of capture. All adults resumed nest attendance 5–10 min after release. Data collection and blood sampling protocols comply with the current laws and ethical standards of animal research in Mexico (NOM-059-SEMARNAT-2010) and were revised and approved by the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT; permit number SGPA/DGVS/01216617). In addition, we confirm that all methods are reported in accordance with ARRIVE guidelines 2.0 (https://arriveguidelines.org).

Parasite identification

Blood smears were scanned for the presence of blood parasites using a light microscope Nikon Eclipse Ti - Arcturus XT of Applied Biosystems. Half of each blood smear was scanned at 400x magnification in search of larger parasites, including *Leucocytozoon*, during 30–45 min. The other half of each smear was scanned at 1000x magnification in search of *Haemoproteus*, *Plasmodium* and *Leucocytozoon* parasites in up to > 10,000 erythrocytes per smear (see [34]) during 30 min. DNA from blood samples were extracted using the Maxwell®16 LEV system Research (Promega, Madison, WI) [35]. Samples were run in 0.8% agarose gels to check DNA integrity. To detect and identify avian parasites of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, we carried out nested PCRs following Hellgren et al. [36] to amplify a fragment of the mitochondrial cytochrome b gene. To check if the PCRs amplified successfully, we ran 3.0 µl of the final PCR product on a 2% agarose gel. We ran 6 repetitions of each PCR to exclude false negatives.

Declarations

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Author contributions

SA and HD conceived the study. FRZ and SA sampled birds. FRZ, JMP, MJRL and JF analyzed the samples. SA drafted the manuscript with significant contributions of the other authors. All authors read and approved the manuscript.

Competing interests

The authors declare no competing interests.

Data availability

Data supporting the conclusions are included within the article.

Ethics approval

Our research complies with Mexican legal and ethical requirements. Permit to collect blood samples (SGPA/DGVS/01216617) was provided by Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT).

Consent for publication

Not applicable.

References

1. Atkinson, C. T. & Van Riper, C. Pathogenicity and epizootiology of avian haematozoa: Plasmodium, Leucocytozoon, and Haemoproteus.*Bird-parasite Interact.*19–48(1991).
2. Sorci, G. & Moller, A. P. Comparative evidence for a positive correlation between haematozoan prevalence and mortality in waterfowl. *J. Evol. Biol*, **10**, 731–741 (1997).
3. Merino, S., Moreno, J., Sanz, J. J. & Arriero, E. Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (Parus caeruleus). Proc. R. Soc. B Biol. Sci. 267, 2507–2510(2000).
4. Asghar, M. *et al.* Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science (80-.)*, **347**, 436–438 (2015).
5. Quillfeldt, P., Arriero, E., Martínez, J., Masello, J. F. & Merino, S. Prevalence of blood parasites in seabirds - a review. *Front. Zool*, **8**, 26 (2011).

6. Piersma, T. Do Global Patterns of Habitat Use and Migration Strategies Co-Evolve with Relative Investments in Immunocompetence due to Spatial Variation in Parasite Pressure?, **80**, 623 (1997).
7. Mendes, L., Piersma, T., Lecoq, M., Spaans, B. & Ricklefs, R. E. Disease-limited distributions? Contrasts in the prevalence of avian malaria in shorebird species using marine and freshwater habitats., **109**, 396–404 (2005).
8. Martínez-Abraín, A., Esparza, B. & Oro, D. Lack of blood parasites in bird species: Does absence of blood parasite vectors explain it all? in Ardeola vol. 51 225–232(2004).
9. Campioni, L. *et al.* Absence of haemosporidian parasite infections in the long-lived Cory's shearwater: evidence from molecular analyses and review of the literature. *Parasitol. Res*, **117**, 323–329 (2018).
10. Osorio-Beristain, M. & Drummond, H. Non-aggressive mate guarding by the blue-footed booby: A balance of female and male control. *Behav. Ecol. Sociobiol*, **43**, 307–315 (1998).
11. Nelson, J. B. *Pelicans, cormorants and their relatives: The Pelecaniformes* (Oxford University Press, 2006).
12. Kim, S. Y., Torres, R., Domínguez, C. A. & Drummond, H. Lifetime philopatry in the blue-footed booby: A longitudinal study. *Behav. Ecol*, **18**, 1132–1138 (2007).
13. Drummond, H. & Rodríguez, C. Viability of booby offspring is maximized by having one young parent and one old parent. *PLoS One*, **10**, e0133213 (2015).
14. Lee-Cruz, L. *et al.* Prevalence of Haemoproteus sp. in Galápagos blue-footed boobies: effects on health and reproduction.*Parasitol. Open***2**, (2016).
15. Santiago-Alarcon, D., Palinauskas, V. & Schaefer, H. M. Diptera vectors of avian Haemosporidian parasites: Untangling parasite life cycles and their taxonomy. *Biol. Rev*, **87**, 928–964 (2012).
16. Bond, J. G. *et al.* Diversity of mosquitoes and the aquatic insects associated with their oviposition sites along the Pacific coast of Mexico. *Parasites and Vectors*, **7**, 1–19 (2014).
17. Ibañez-Bernal, S. Informe Final del proyecto Actualización del Catálogo de Autoridad Taxonómica del orden Diptera (Insecta) de México CONABIO (JE006)(2017).
18. Levin, I. I. *et al.* Hippoboscid-transmitted Haemoproteus parasites (Haemosporida) infect Galapagos Pelecaniform birds: Evidence from molecular and morphological studies, with a description of Haemoproteus iwa. *Int. J. Parasitol*, **41**, 1019–1027 (2011).
19. Madsen, V. *et al.* Testosterone levels and gular pouch coloration in courting magnificent frigatebird (Fregata magnificens): Variation with age-class, visited status and blood parasite infection. *Horm. Behav*, **51**, 156–163 (2007).
20. Clark, G. W. & Swinehart, B. Avian haematozoa from the offshore islands of northern Mexico. *Wildl. Dis*, **5**, 111–112 (1969).
21. Quillfeldt, P. *et al.* Hemosporidian blood parasites in seabirds - A comparative genetic study of species from Antarctic to tropical habitats., **97**, 809–817 (2010).
22. Merino, S. *et al.* Infection by haemoproteus parasites in four species of frigatebirds and the description of a new species of Haemoproteus (Haemosporida: Haemoproteidae). *J. Parasitol*, **98**,

388–397 (2012).

23. Svensson, L. M. E. & Ricklefs, R. E. Low diversity and high intra-island variation in prevalence of avian Haemoproteus parasites on Barbados, Lesser Antilles., **136**, 1121–1131 (2009).
24. Loiseau, C. *et al.* Spatial variation of haemosporidian parasite infection in african rainforest bird species. *J. Parasitol*, **96**, 21–29 (2010).
25. Madsen, V. *Female mate choice in the magnificent frigatebird (Fregata magnificens)* (Universidad Nacional Autónoma de México, 2004).
26. Super, P. E. & van Riper, C. A comparison of avian hematozoan epizootiology in two California coastal scrub communities. *J. Wildl. Dis*, **31**, 447–461 (1995).
27. CONANP. *Programa de Conservación y Manejo del Parque Nacional Isla Isabel*. (2005).
28. Ancona, S., Drummond, H., Rodríguez, C. & Zúñiga-Vega, J. J. Long-term population dynamics reveal that survival and recruitment of tropical boobies improve after a hurricane. *J. Avian Biol*, **48**, 320–332 (2017).
29. Ancona, S., Sánchez-Colón, S., Rodríguez, C. & Drummond, H. El Niño in the Warm Tropics: Local sea temperature predicts breeding parameters and growth of blue-footed boobies. *J. Anim. Ecol*, **80**, 799–808 (2011).
30. Anderson, D. J., Huyvaert, K. P. & Anchundia, D. Chronic lack of breeding by Galápagos Blue-footed Boobies and associated population decline. Galapagos Report 2013–*2014* vol. 9 https://www.galapagos.org/wp-content/uploads/2015/09/GalapagosReport_2013-2014-22-Anderson-158-163.pdf (2015).
31. Valkiunas, G. *et al.* A comparative analysis of microscopy and PCR-based detection methods for blood parasites. *J. Parasitol*, **94**, 1395–1401 (2008).
32. Santiago-Alarcon, D. *et al.* Parasites in space and time: a case study of haemosporidian spatiotemporal prevalence in urban birds. *Int. J. Parasitol*, **49**, 235–246 (2019).
33. Drummond, H., Torres, R. & Krishnan, V. V. Buffered development: Resilience after aggressive subordination in infancy. *Am. Nat*, **161**, 794–807 (2003).
34. Merino, S. & Potti, J. High Prevalence of Hematozoa in Nestlings of a Passerine Species, the Pied Flycatcher (Ficedula hypoleuca). *Auk*, **112**, 1041–1043 (1995).
35. Gutiérrez-López, R. *et al.* Low prevalence of blood parasites in a long-distance migratory raptor: The importance of host habitat. *Parasites and Vectors*, **8**, 1–6 (2015).
36. Hellgren, O., Waldenström, J. & Bensch, S. A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. *J. Parasitol*, **90**, 797–802 (2004).