

# LOW PREVALENCE OF *PLASMODIUM* AND *HAEMOPROTEUS* IN RESIDENT SEABIRDS FROM OCEANIC ISLANDS OFF THE BRAZILIAN COAST

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## ABSTRACT

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Bird pathogens promote morbidity and mortality that affect bird population stability. In this context, the present study aimed to detect blood intracellular parasites by molecular methods in four species of seabirds, Masked Booby *Sula dactylatra*, Red-footed Booby *S. sula*, Brown Booby *S. leucogaster*, and Magnificent Frigatebird *Fregata magnificens*, on the Brazilian coast. Blood samples were collected in the Archipelagos of São Pedro and São Paulo, Fernando de Noronha, Atol das Rocas, and Trindade and Martim Vaz. Genomic DNA was extracted with proteinase K digestion and purification with phenol and chloroform. For each sample, three independent polymerase chain reactions were performed with a positive control for *Plasmodium* and *Haemoproteus*. Corroborating other studies, our results indicate a low prevalence of *Plasmodium* and *Haemoproteus* parasitemia in this group of birds in the wild. We propose that the low parasitemia occurred due to 1) the absence of the vector for transmission among individuals of these islands; 2) the low number of parasitized individuals detected in the colonies sampled; and 3) an effective immune response that prevented installation of the parasite. Only the Masked Booby and Magnificent Frigatebird have sufficient flight capacity to reach the Brazilian coast, but Masked Boobies rarely visit waters near the coast. Without vector studies at the archipelagos that were sampled, these hypotheses cannot be assessed.

**Key words:** *Plasmodium*, *Haemoproteus*, seabirds, blood parasites, South Atlantic Ocean

## INTRODUCTION

Blood parasites having the highest rate of occurrence in birds are grouped in the *Babesia*, *Leucocytozoon*, *Trypanosoma*, and *Hepatozoon* genera. *Plasmodium* and *Haemoproteus* (Haemosporida, Apicomplexa) are considered the most common blood parasites in birds, causing avian malaria (Quillfeldt *et al.* 2013, Sallaberry-Pincheira *et al.* 2015). These pathogens promote high morbidity and mortality and are considered important in avian conservation, including seabird conservation (Vanstreels *et al.* 2016a). Avian malaria has been reported in 22 orders of birds distributed in the Holarctic, Neotropical, Afro-tropical, Indo-Malaysian, and Australian regions; the disease has not been recorded in the Antarctic, apparently because low temperatures make it difficult for the host to survive (Vanstreels *et al.* 2014).

High parasitemia may affect host reproduction or even lead to death (Quillfeldt *et al.* 2013). However, reductions in host population size tends to lead to reductions in parasite numbers. In this way, the host-parasite interaction has co-evolved, creating an equilibrium whereby the parasite expresses a reduction in the adaptive value of the host by exhibiting a great capacity for infection, and the host uses efficient immunological mechanisms to reduce the adaptive value of the parasite (Anderson & Gordon 1982, Ridley 2006). However, anthropogenic activities have caused environmental disequilibrium, increasing host susceptibility to infectious agents (Laurance *et al.* 2013). Other factors that have increased the susceptibility to infection include a loss of genetic diversity due to endogamy, which reduces the immunological capacity of organisms to respond to new pathogens (Brearley *et al.* 2012). Thus, host and

vector population monitoring are important to the conservation of biodiversity and control of diseases in wild birds.

Malaria affects island avifaunas around the world due to introduced, infected mosquitos (Liao *et al.* 2015, Olsson-Posson *et al.* 2015, Yabsley *et al.* 2018). Only 113 seabirds have been investigated for disease, and 31 species (27%) showed hematozoary presence (Quillfeldt *et al.* 2011). In South America, only a few seabird species have been studied. Sallaberry-Pincheira *et al.* (2015) evaluated Humboldt *Spheniscus humboldti* and Magellanic penguins *S. magellanicus* at 13 breeding colonies in Peru, Chile, and in Argentina, and found low avian malaria prevalence, i.e., only three of 861 individuals were positive for malaria. Quillfeldt *et al.* (2013) studied three species of boobies (Masked Booby *Sula dactylatra*, Brown Booby *S. leucogaster*, and Red-footed Booby *S. sula*) and found no individuals infected by avian malaria. In this context, the present study evaluated the parasitemia of avian malaria through molecular techniques in four species of seabirds—Masked Booby, Red-footed Booby, Brown Booby, and Magnificent Frigatebird *Fregata magnificens*—on the Brazilian coast.

## METHODS

Our study involved three sulids, Masked Booby, Brown Booby, and Red-footed Booby, and one fregatid, Magnificent Frigatebird. Boobies are widely distributed in tropical regions, often occurring well off the coast. They exhibit gregarious behavior, nest on land or vegetation, and feed on fish and squid occurring close to the surface. Magnificent Frigatebirds occur in tropical and subtropical seas, are mostly coastal, and feed, in part, by kleptoparasitism (Osorno *et al.* 1992).

### Sampling

Blood samples ( $n = 233$ ; Table 1) were collected during 2015 and 2016 in four archipelagos on the Brazilian coast (Fig. 1): Fernando de Noronha, Atol das Rocas, São Pedro and São Paulo, and Trindade and Martim Vaz. Blood was taken from the brachial vein (~1 mL) with 3-mL syringes and 23G needles and was stored in 70% ethanol. DNA was then extracted from these samples by proteinase K digestion and was purified with phenol and chloroform (Sambrook *et al.* 2001).

### Sample Analysis

*Plasmodium* and *Haemoproteus* detection was performed by Polymerase Chain Reaction (PCR), using four primers previously described for detection of avian hemoparasites: HAEMF (5'-ATGGTGCCTTCGATATATGCATG-3'), HAEMR2

(5'-GCATTATCTGGATGTGATAATGGT-3') (Hellgren *et al.* 2004); 3760F (5'-GAGTGGATGGTGTTTTAGAT-3'), 4292RW2 (5'-TGGAACAATATGTARAGGAGT-3') (Beadell *et al.* 2004). PCR was performed in a 10  $\mu$ L reaction containing 50 ng of DNA, 2.5 mM MgCl<sub>2</sub>, 2.5 mM dNTPs, 6  $\mu$ M of each primer, 1 $\times$  Buffer, and 0.5 U of GoTaq® Flexi DNA Polymerase (Promega). The following thermal cycle was used: 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, annealing temperature at 50 °C for 30 s, 72 °C for 45 s, and final extension at 72 °C for 10 min. PCR products were separated by gel electrophoresis in a 2% agarose gel for 20 min at 80 volts. For all PCRs a positive control for *Plasmodium* and/or *Haemoproteus* was included. Each sample was subjected to at least three PCRs independently in order to confirm the positive or negative diagnosis for malaria. Successful amplification of a PCR product was considered a positive result and non-amplification was considered a negative result.

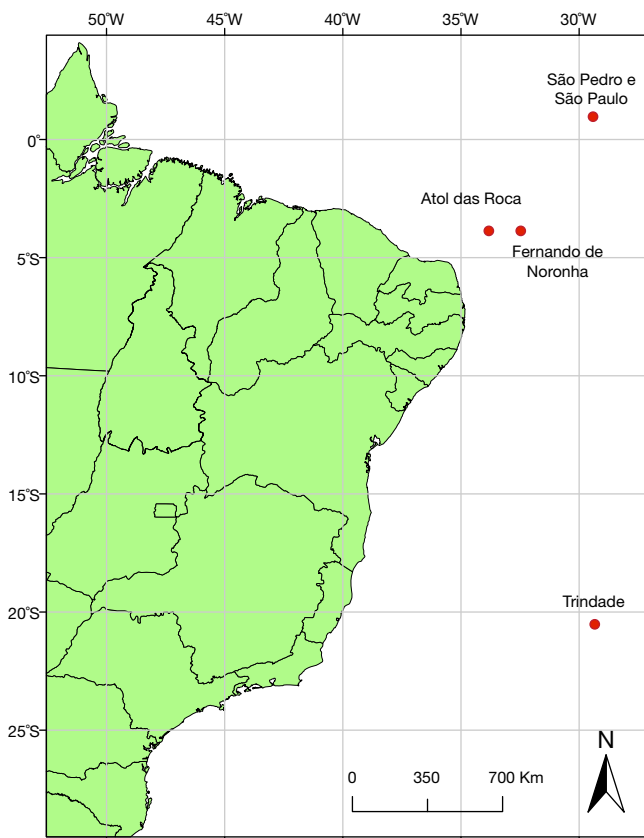
### RESULTS

We did not detect blood parasites (*Plasmodium*/*Haemoproteus*) in any of the individuals analyzed, indicating that avian malaria is not present in Masked Boobies, Red-footed Boobies, Brown Boobies, and Magnificent Frigatebirds in the study area (Table 1).

### DISCUSSION

The low prevalence of avian malaria observed in the four species of seabirds from islands off the Brazilian coast may be related to environmental conditions that affect vector survival, as well as the seabirds' dispersal ability, high genetic diversity, and efficient immune systems. Low parasitemia has yet to be reported for boobies and penguins (Quillfeldt *et al.* 2011, Quillfeldt *et al.* 2013, Sallaberry-Pincheira *et al.* 2015), so we have formulated some hypotheses to explain this pattern. One explanation for the low prevalence of avian malaria in our study is that the environmental conditions of the Brazilian oceanic islands do not favor the natural occurrence of vectors for parasite dissemination. *Aedes* mosquitos are a known vector for avian malaria, and in Fernando de Noronha between 1984 and 2017, there were 365 cases of dengue transmitted by *Aedes* mosquitos (Ministério de Saúde-Brasil, 2016). However, no records of vectors exist for other oceanic islands off this coast.

A second hypothesis to explain the low prevalence of avian malaria in Brazilian oceanic seabirds relates to seabird dispersal ability. This hypothesis assumes that avian species that disperse widely could be exposed to infection away from breeding sites as they visit other locations where vectors are present, leading to parasitic infection. For instance, Vanstreels *et al.* (2020) found an individual Manx shearwater *Puffinus puffinus*, a migratory species, that was infected by avian malaria. The bird was visiting waters off the



**Fig. 1.** Eastern coast of Brazil showing oceanic islands where samples were obtained.

**TABLE 1**  
Localities with respective number of individuals sampled and percentage of avian malaria detected

Archipelago		Red-footed Booby <i>Sula sula</i>	Brown Booby <i>Sula leucogaster</i>	Masked Booby <i>Sula dactylatra</i>	Magnificent Frigatebird <i>Fregata magnificens</i>
São Pedro and São Paulo	ASP	-	34 (0%)	-	-
Atol das Rocas	ATR	30 (0%)	22 (0%)	36 (0%)	-
Fernando de Noronha	NOR	12 (0%)	13 (0%)	31 (0%)	13 (0%)
Trindade e Martim Vaz	TRI	-	-	25 (0%)	-

Brazilian coast, and the infection likely occurred during migration. Coastal seabirds can show a high prevalence of malaria, as observed in Caspian Gull *Larus cachinnans* along the coast of Poland (93% prevalence; Zagalska-Neubauer & Bensch 2016).

Ecological data show that Brown Boobies can disperse up to 129 km (Weimerskirch *et al.* 2009). However, Nunes *et al.* (2017) observed that in the São Pedro and São Paulo Archipelago, birds of this species were absent from the island for only 57 min, traveling a maximum distance of 27 km. Thus, this species appears to be mostly resident where it nests in the study area. It has been reported that Red-footed and Masked boobies disperse up 114 km and 900 km, respectively (Blaber *et al.* 1995, Weimerskirch *et al.* 2005). On the other hand, Magnificent Frigatebirds are long-distance travelers, reaching ~1341 km from breeding sites (Weimerskirch *et al.* 2006, Rattenborg *et al.* 2016). Thus, only Magnificent Frigatebirds and Masked Boobies would be likely to travel between the continent and the oceanic islands.

Alternatively, the low prevalence or absence of malaria in Brazilian oceanic seabirds may be due to their high genetic diversity or their efficient of immune systems, which allow them to evade parasitic infection. Sallaberry-Pincheira *et al.* (2016) observed that Humboldt and Magellan penguin colonies exhibit high genetic diversity for the Major Histocompatibility Complex (MHC), so the evolutionary and adaptive potential of species facing an infectious disease depends on their genetic diversity in MHC-related genes. The only study done on Magnificent Frigatebirds of the Brazilian coast found little genetic diversity using mtDNA (NDH2 and Cytb) and microsatellites (Nuss *et al.* 2016). In addition, Nunes & Bugoni (2018) also observed low genetic diversity in Brown Boobies from the São Pedro and São Paulo archipelago. Studies that evaluate MHC diversity and parasitic infections are otherwise scarce or inconclusive (Sutton *et al.* 2016). Two studies of Magellanic Penguins showed that avian malaria was absent in colonies (Sallaberry-Pincheira *et al.* 2016), but that it was present at a prevalence of 7% in captive penguins (Vanstreels *et al.* 2014), raising the possibility that immunosuppression in captivity could favor infection.

In conclusion, avian malaria was not present in oceanic seabird species from the western South Atlantic Ocean. However, it is extremely important to monitor oceanic islands to avoid dissemination of this vector. Increasing levels of precipitation and rising temperatures related to climate change, as well as increasing tourism, leave these oceanic islands vulnerable to invasion by malaria.

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