MOLECULAR SCREENING OF AVIAN VIRUSES AND PARASITES IN WEDGE-TAILED SHEARWATERS *ARDENNA PACIFICA* AND BARAU'S PETRELS *PTERODROMA BARAUI* ON RÉUNION ISLAND

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ABSTRACT

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We investigated Wedge-tailed Shearwater *Ardenna pacifica* and Barau's Petrel *Pterodroma baraui* exposure to infectious agents circulating in the western Indian Ocean. Using polymerase chain reaction and serological analyses, we screened for influenza viruses, flaviviruses, coronaviruses, and blood parasites (*Haemoproteus*, *Leucocytozoon*, and *Plasmodium*). No active infections were detected and serological results suggested limited past exposure, with only two Wedge-tailed Shearwaters testing positive for antibodies to the influenza A virus. These findings indicate the limited role of these species as epidemiological reservoirs. However, environmental factors such as tick infestations and isolated breeding habitats highlight the need for continued epidemiological monitoring to support effective conservation strategies.

Key words: blood parasites, coronavirus, flavivirus, influenza viruses, Procellariiformes, Indian Ocean

INTRODUCTION

The order Procellariiformes contains many endangered species (Dias et al., 2019). These seabirds are significantly affected by anthropogenic changes, including the introduction of invasive species at breeding sites (Spatz et al., 2022), bycatch in fisheries, depletion of their prey (Paleczny et al., 2015; Grémillet et al., 2018), and light pollution (Rodríguez et al., 2019). Knowledge of the degree to which petrels are affected by infectious agents remains limited. This gap is likely due to their complex life cycle, which includes an extensive pelagic phase during which contact with researchers is very low. During their terrestrial breeding phase, they nest in cavities, crevices, and burrows, often on remote and inaccessible islands, which limits access even further. Although diseases have been described (e.g., puffinosis, which may be caused by a coronavirus; Nuttall & Harrap, 1982), the diversity and drivers of pathogen transmission in petrels and shearwaters remain poorly understood (Rodríguez et al., 2019).

Several infectious agents have been identified in seabirds of the western Indian Ocean, including low-pathogenic influenza viruses (Lebarbenchon et al., 2015, 2023), flaviviruses (potentially transmitted by ticks; Jaeger et al., 2016), and blood parasites (Bastien et al., 2014). The circulation of these pathogens in seabird populations has been demonstrated through both the direct detection of infectious agents and serological analyses. Serological testing can also reveal past exposure and the development of specific immune responses. This combined approach of integrating molecular detection with serology provides a complete picture of exposure levels to targeted infectious agents. Such information is crucial for assessing the potential role of seabird species as epidemiological reservoirs.

The objective of this study was to investigate both past exposure and active circulation of infectious agents commonly detected in Procellariiformes of the western Indian Ocean. We focused on two species from Réunion Island: the widespread Wedge-tailed Shearwater *Ardenna pacifica* and the endemic Barau's Petrel *Pterodroma baraui*. Our methodology involved the direct detection of influenza viruses, flaviviruses, coronaviruses, and parasites (*Haemoproteus*, *Leucocytozoon*, and *Plasmodium*), using polymerase chain reactions (PCR). In addition, serological analyses were conducted to identify evidence of past infections to influenza, West Nile, and infectious bronchitis viruses. This research aligns with ongoing conservation efforts for these two species on Réunion Island (Faulquier et al., 2017; Virion et al., 2021).

METHODS

Repeated sampling of Wedge-tailed Shearwaters was conducted between 2014 and 2018 at the Grande Anse and Petite Île colonies on the southern coast of Réunion Island (21.373°S, 055.55°E), where approximately 200 breeding pairs gather (Choeur et al., 2022). Only one sampling session was performed for Barau's Petrels at the Bras des Étangs colony (21.105°S, 055.475°E) due to the challenging accessibility of nesting sites and logistical constraints related to the preservation of biological samples. The petrel colony is in the central region of the island at an altitude of 2,500 m above sea level and comprises approximately 180 breeding pairs (Virion et al., 2021). For both species, only breeding adults were included in the study.

Cloacal swabs were collected from 131 birds using sterile rayon-tipped applicators (Puritan; Guilford, USA) and placed in a tube containing 1.5 mL of viral transport medium. Samples were maintained at 4 °C in the field and stored at −80 °C at the lab until further analysis. Blood samples (up to 1% of bird body weight) were also collected from all 131 birds; plasma was separated by centrifugation and stored at −20 °C. All birds were banded. Procedures related to the capture, handling, and collection of biological materials were approved by the Center for Research on Bird Population Biology (National Museum of Natural History in Paris, France; Personal Program Nos. 609 and 616) and by the French Ministry of Education and Research (APAFIS#3719-2016012110233597v2).

For PCR analysis, swabs were centrifuged at 1,500 g for 15 min, and RNA was extracted from 140 µL of the supernatant using the QIAamp Viral RNA Mini Kit (QIAGEN; Valencia, USA). Nucleic acids were extracted from 200 µL of red blood cells using the QIAamp cador Pathogen Mini Kit (QIAGEN). Reverse transcription was performed following the protocol described by Lebarbenchon et al. (2017). Molecular detection of influenza viruses, coronaviruses, flaviviruses, and blood parasites was conducted according to Spackman et al. (2002), Moureau et al. (2007), Muradrasoli et al. (2009), and Hellgren et al. (2004), respectively. PCR assays were carried out using the QuantiNova Probe PCR Master Mix (QIAGEN) for influenza viruses and coronaviruses, and the GoTaq G2 Hot Start Green Master Mix (Promega; Madison, USA) for flaviviruses and blood parasites. Prior to RNA extraction, 10 µL of MS2 bacteriophage RNA was added to each sample as an internal control. All samples were subsequently tested for MS2 phage cDNA to validate the efficiency of the extraction and reverse transcription steps (Ninove et al., 2011).

Antibodies against avian influenza virus (AIV), West Nile virus (WNV), and infectious bronchitis virus (IBV) were detected using enzyme-linked immunosorbent assays (ELISAs), specifically the ID Screen Influenza A Antibody Competition Multi-Species, West Nile Competition Multi-Species, and Infectious Bronchitis Competition kits (IDvet; Grabels, France). An optimized protocol for the detection of antibodies against influenza viruses was applied, as described by Lebarbenchon et al. (2012). Specificity of the WNV assay was limited, as we previously identified specific antibodies against Usutu and Meaban viruses by neutralisation tests in ELISA-positive samples (Jaeger et al., 2016). Finally, although we tested samples with the Infectious Bronchitis Competition assay, no information regarding its specificity to avian coronavirus was available. Due to limited plasma volumes, samples were first tested for AIV (*n* = 131), followed by WNV (*n* = 126), and IBV (*n* = 114).

RESULTS AND DISCUSSION

Influenza viruses, flaviviruses, coronaviruses, and blood parasites (*Haemoproteus*, *Leucocytozoon*, *Plasmodium*) were not detected in cloacal swabs or red blood cell samples, suggesting that these pathogens were not circulating in Wedge-tailed Shearwater and Barau's Petrel populations at the time of sampling (Table 1). Serological analyses further indicated that the tested birds had no prior exposure to IBV or WNV (Table 2). However, two Wedge-tailed Shearwaters tested positive for antibodies against influenza viruses (Table 2). Due to the limited volume of plasma, influenza virus subtyping was not performed.

These results confirm the limited role of Procellariiformes as reservoirs for influenza viruses in the western Indian Ocean, particularly when compared to terns and noddies (Laridae), where seroprevalence can exceed 60% (Lebarbenchon et al., 2023). In the case of Wedge-tailed Shearwaters, a previous study reported a seroprevalence of 5.4% in the same population in December 2012 (Lebarbenchon et al., 2015). The two AIV-positive birds identified in our study were sampled on an islet where shearwaters breed sympatrically with Brown Noddies *Anous stolidus.* Also living at this colony is a roost of non-breeding Lesser Noddies *Anous tenuirostris*, in which AIV of the H2 subtype was detected in 2013 (Lebarbenchon et al., 2015). The limited seroprevalence estimated in 2016 for the current study (6%) suggests that influenza viruses do not circulate continuously within this species on Réunion Island. Instead, seropositivity was likely the result of sporadic infections from viruses circulating in noddy populations. This seroprevalence is also consistent with those reported for this species in other studies (see review by Lang et al., 2016).

The absence of vector-borne viruses and blood parasites in our study contrasts with findings from other seabird species in the western Indian Ocean. Flavivirus seroprevalence exceeding 70% has been reported in Masked Boobies *Sula dactylatra* on Tromelin

TABLE 1
Detection of influenza virus, flavivirus, coronavirus, and blood parasites (*Haemoproteus*, *Leucocytozoon*, and *Plasmodium*) via polymerase chain reaction in Wedge-tailed Shearwaters *Ardenna pacifica* and Barau's Petrels *Pterodroma baraui*. Values shown are the number of positive birds / number of birds tested.

Species	Year	Influenza A virus	Flavivirus	Coronavirus	*Haemoproteus, Leucocytozoon, Plasmodium* parasites
Barau's Petrel	2018	0/31	0/31	0/31	0/31
Wedge-tailed Shearwater	2014	0/28	0/28	0/28	0/28
	2015	0/25	0/25	0/25	0/25
	2016	0/33	0/33	0/33	0/33
	2018*	0/14	0/14	0/14	0/14

* Includes one sample collected early January 2019.

TABLE 2
Detection of antibodies to influenza, West Nile, and infectious bronchitis viruses via enzyme-linked immunosorbent assay in Wedge-tailed Shearwaters *Ardenna pacifica* and Barau's Petrels *Pterodroma baraui*. Values shown are the number of positive birds / number of birds tested.

Species	Year	Influenza A virus	West Nile virus	Infectious bronchitis virus
Barau's Petrel	2018	0/31	0/26	0/21
Wedge-tailed Shearwater	2014	0/28	0/28	0/26
	2015	0/25	0/25	0/24
	2016	2/33	0/33	0/29
	2018*	0/14	0/14	0/14

* Includes one sample collected early January 2019.

Island (500 km north of Réunion Island; Jaeger et al., 2016), while *Haemoproteus iwa* and *Plasmodium* spp. parasites have been detected in Great Frigatebirds *Fregata minor* on Europa Island in Mozambique Channel (Bastien et al., 2014). On Réunion Island, heavy infestations by the ticks *Amblyomma loculosum* and *Ornithodoros capensis* have also been documented in Wedge-tailed Shearwaters (Le Rouzic, 2013), suggesting the potential for transmission of vector-borne viruses in this species.

Differences in exposure to vector-borne viruses and blood parasites may also be influenced by the migratory strategies of the species in question. For instance, noddies undertake partial migrations and frequently aggregate at roosts during migration, facilitating intra-specific transmission (Lebarbenchon et al., 2023). Similarly, frigatebirds use roosting sites during migration (Weimerskirch et al., 2017), increasing the potential for parasite exposure. In contrast, Barau's Petrels and Wedge-tailed Shearwaters remain at sea for extended periods during migration, i.e. for several consecutive months (Catry et al., 2009; Pinet et al., 2011). While these behavioral differences do not fully explain the observed patterns of exposure, the relationship between migratory strategies and infection dynamics warrants further investigation (Altizer et al., 2011).

The absence of directly transmitted viruses in Barau's Petrels may also be attributed to their distinct ecology compared to Wedge-tailed Shearwaters. Barau's Petrels occupy remote and isolated terrestrial habitats that are not shared with other seabird species, thereby limiting opportunities for inter-species transmission. Furthermore, the lack of ticks and other arthropod vectors reduces the risk of arbovirus and blood-parasite transmission on their breeding grounds (personal observations by CL, 2018). However, given the limitations of our sampling in terms of sample size, population coverage, and temporal frame, these interpretations should be approached with caution. This is particularly important considering the unpredictability of seabird exposure to emerging zoonoses, as evidenced by the global spread of the H5N1 AIV.

This study provides novel insights into the exposure to viruses and blood parasites of two seabird species from the southwestern Indian Ocean. Although most results were negative, these findings are significant as they offer a baseline assessment of the circulation of potentially pathogenic agents in seabirds exposed to multiple environmental threats. The poor reproductive success of Wedge-tailed Shearwaters on Réunion Island is mostly due to predation by feral Domestic Cats *Felis catus* (Choeur et al., 2022). Heavy infestations of chicks by ticks could also account for the limited breeding success, particularly on the small islet of Petite Île, and this might also be related to the transmission of *Rickettsia* spp. and *Coxiella* spp. bacteria by *A. loculosum* and *O. capensis* ticks (Dietrich et al., 2014; Wilkinson et al., 2014). Overall, the collection and integration of epidemiological data are essential for identifying the range of threats facing these species and for implementing appropriate conservation strategies.

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AUTHOR CONTRIBUTIONS

Conceptualization: CL. Investigation: CL, CT, SB, AJ, MLC. Project administration: CL, MLC. Writing – original draft: CL. Writing – review and editing: SB, MLC, AJ.

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