ABSENCE OF CORONAVIRUS IN TERNS AND NODDIES IN THE WESTERN INDIAN OCEAN?

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ABSTRACT

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We investigated coronavirus circulation in three tern species on four islands of the Western Indian Ocean (Bird, Reunion, Europa, Juan de Nova). None of the 2019 samples tested positive by reverse-transcription polymerase chain reaction. We discuss the implications of these findings in terms of host-species range, ecological drivers of virus transmission, and diagnostic tools.

Key words: *Anous stolidus, Anous tenuirostris, Onychoprion fuscatus*, Seychelles, Reunion Island, Eparses Islands

Bats and birds are natural hosts for Coronavirus (CoV; Coronaviridae). The emergence of bat-related CoV in humans has stimulated research on these viruses for the past decade, but knowledge of the eco-epidemiology and evolution of bird-borne CoVs (gamma- and delta-CoV) remain limited (Wille & Holmes, 2020). CoVs have been detected in wild birds on all continents, but a major sampling bias toward wild ducks has led to uneven reports of virus prevalence and diversity between bird taxa (see Wille & Holmes, 2020 for a review). Indeed, a limited number of studies have investigated CoV infections in seabirds, although both gamma- and delta-CoV were detected in gulls and shorebirds (Wille & Holmes, 2020). Clinical signs of disease associated with these viruses have not been reported, but the consequences of seabird infections with poultry-associated viruses, such as infectious bronchitis virus (IBV), need to be considered. The emergence and intercontinental spread of the highly pathogenic H5N1 avian influenza virus (AIV), which originated from poultry, has been responsible for unprecedented mass mortality in seabirds. In this context, spillover and emergence risk of other avian viruses, such as IBV, should be fully considered. Further epidemiological studies are needed to assess CoV host-species range in seabirds in order to identify the ecological drivers of viral infection and the risk of spillover potential to poultry.

Spatial isolation could represent a major barrier to the natural introduction of infectious agents on oceanic islands, but that same isolation may also generate ecological conditions favoring the local maintenance of viruses in wild bird communities inhabiting these islands. In the Western Indian Ocean, oceanic islands are major breeding sites for seabirds, with several species aggregating at very high densities. Most of these species are pelagic; migratory gulls and ducks do not breed or roost on these islands. We previously identified Brown Noddies *Anous stolidus* and Lesser Noddies *A. tenuirostris* (Charadriiformes) as major AIV hosts and, to a lesser extent, Sooty Terns *Onychoprion fuscatus* (Lebarbenchon et al., 2015). Lebarbenchon et al. (2013, 2015) found major differences between taxa in the prevalence of birds testing positive and seropositive for AIV, suggesting species-specific variation in virus circulation. CoVs were also screened in more than 300 cloacal swabs collected from eight seabird species, mostly from species of Phaethontiformes, Procellariiformes, and Suliformes, but none tested positive for the presence of CoV RNA (Lebarbenchon et al., 2013).

We investigated CoV circulation in three Charadriiformes species—Brown Noddy, Lesser Noddy, and Sooty Tern—on four islands of the Western Indian Ocean. Bird Island, the northernmost island of the Seychelles archipelago (03°43′S, 055°12′E), is a major breeding site for terns, with approximately 400,000 pairs of Sooty Terns, *ca*. 10,000 pairs of Brown Noddies, and *ca*. 19,000 pairs of Lesser Noddies (Feare, 1976, 1979). Europa Island (22°23′S, 040°21′E) and Juan de Nova Island (17°03′S, 042°43′E), located in Mozambique Channel, host two major Sooty Tern colonies (760,000 and 2,000,000 breeding pairs, respectively; Le Corre & Jaquemet, 2005). Small populations (hundreds to several thousands) of Brown and Lesser noddies breed and roost on Reunion Island (21°22′S, 055°34′E).

We searched for the presence of CoV RNA in samples previously collected and tested for AIV (N = 1,459; Lebarbenchon et al., 2015, 2023), as well as in other samples (n = 560) from Brown Noddies and Lesser Noddies on Reunion Island (Table 1). Only adult birds were included in the study. For each bird, cloacal and oropharyngeal swabs were collected and placed in a single tube containing 1.5 ml of Brain Heart Infusion media (Condalab, Madrid, Spain) supplemented with penicillin G (1,000 units/ml), streptomycin

TABLE 1
Number of cloacal and oropharyngeal samples tested for coronavirus RNA in the Western Indian Ocean, per tern species, island, and year

Species	Island	Month/Year	*N* tested samples
Brown Noddy *Anous stolidus*			
	Bird	June 2012	33
		June 2013	90
		June–July 2014	144
		July 2015	133
		July 2017	162
		July 2018	164
		July 2019	36
	Reunion	November 2016	31
Lesser Noddy *Anous tenuirostris*			
	Bird	June 2012	32
		June 2013	90
		June–July 2014	141
		July 2015	101
		July 2017	58
		July 2018	35
		July 2019	10
	Reunion	March 2013	58
		November 2016	23
Sooty Tern *Onychoprion fuscatus*			
	Bird	June 2012	93
		June 2013	100
		June–July 2014	109
		July 2015	53
		July 2017	31
		July 2018	10
	Europa	July 2012	191
	Juan de Nova	June 2012	91

(1 mg/ml), kanamycin (0.5 mg/ml), gentamicin (0.25 mg/ml), and amphotericin B (0.025 mg/ml). Swabs were maintained at 4 °C in the field, shipped to the laboratory within 48 hours, and held at −80 °C until tested.

Bird capture, bird handling, and the collection of biological material were approved by the Center for Research on Bird Population Biology (National Museum of Natural History, Paris, France), the Seychelles Bureau of Standards, and the Seychelles Ministry of Agriculture, Climate Change and Environment. All procedures were also evaluated and approved by the French Ministry of Education and Research (APAFIS#3719-2016012110233597v2).

Samples were vortexed and centrifuged at 1500 g for 15 minutes. RNA was obtained with the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, USA). Reverse-transcription was performed on 10 µL of RNA using the ProtoScript II Reverse Transcriptase, Random Primer 6, and RNAse inhibitor (New England BioLabs, Ipswich, USA) under the following thermal conditions: 70 °C for 5 minutes, 25 °C for 10 minutes, 42 °C for 50 minutes, and 65 °C for 20 minutes (Lebarbenchon et al., 2017). We tested cDNAs for the presence of the CoV RNA-dependent RNA-polymerase (*RdRp*) gene using a pan-CoV multi-probe real-time polymerase chain reaction (PCR; Muradrasoli et al., 2009) protocol routinely used in our laboratory (Joffrin et al., 2020, 2022; Lebarbenchon et al., 2013). PCRs were performed with the QuantiNova Probe PCR Master Mix (Qiagen, Hilden, Germany) in a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, USA), with positive (Pintail CoV PBA-15 GU393339 and Bat CoV RB369 MN183188) and negative (PCR water) controls. Before RNA extraction, 10 µl of RNA of the MS2 bacteriophage was added to each sample. All samples were then tested for cDNA of the MS2 phage in order to validate the extraction and reverse-transcription steps (Lebarbenchon et al., 2013; Ninove et al., 2011).

None of the 2,019 samples tested positive for the presence of CoV RdRp. This finding is consistent with our previous report that focused on other seabird taxa and was based on a lower number of tested samples (Lebarbenchon et al., 2013). Further investigations are needed into other seabird species and tropical islands to fully decipher the biological and ecological factors involved in a potential spatial restriction in CoV circulation. The development of serological tools could provide additional information and be applied to the detection of gamma- and delta-CoV antibodies, although the immune response to CoV infection remains to be described in seabirds (e.g., waning of CoV-specific antibodies, maternal transfer, cross-reactivity). Seasonality in CoV transmission dynamics as well as differences between bird age-classes could also explain our negative result. Such variation in CoV shedding is suspected in ducks (Wille et al., 2015, 2017) and has been described in bats (e.g., Joffrin et al., 2022) and other host-parasite systems (Altizer et al., 2006). This suggests that further longitudinal studies in seabird populations in the tropics are needed. Finally, epidemiological surveillance of IBV and other avian pathogens circulating among poultry near seabird colonies is critical to mitigate spillover risk.

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