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

CAMILLE LEBARBENCHON[1]*, CHRIS FEARE[2], CHRISTINE LAROSE[2], MARIE-ALICE SIMBI[1], SOLENN BOUCHER[1,3], AUDREY JAEGER[3], & MATTHIEU LE CORRE[3]

[1]*Université de La Réunion, Unité Mixte de Recherche, Processus infectieux en milieu insulaire tropical, Institut national de la santé et de la recherche médicale, Centre National de la Recherche Scientifique, Institut de Recherche pour le Développement, 2 rue Maxime Rivière, Sainte-Clotilde, La Réunion, France *(camille.lebarbenchon@univ-reunion.fr)*

[2]*WildWings Bird Management, Haslemere, Surrey, United Kingdom*

[3]*Université de la Réunion, Unité Mixte de Recherche, Ecologie marine tropicale des océans Pacifique et Indien, Centre National de la Recherche Scientifique, Institut de Recherche pour le Développement, Institut Français de Recherche pour l'Exploitation de la Mer, Université de Nouvelle-Calédonie, 15 avenue René Cassin, Saint-Denis, La Réunion, France*

Received 10 April 2024, accepted 17 August 2024

ABSTRACT

Lebarbenchon, C., Feare, C., Larose, C., Simbi, M.-A., Boucher, S., Jaeger A., & Le Corre, M. (2025). Absence of Coronavirus in terns and noddies in the Western Indian Ocean? *Marine Ornithology, 53*(1), 189–191. http://doi.org/10.5038/2074-1235.53.1.1614

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.

ACKNOWLEDGEMENTS

We are grateful to the Savy family for their warm hospitality and support in the field work on Bird Island. We also thank Matthieu Bastien, Sophie Bureau, Muriel Dietrich, Sébastien Lefort, Bernard Rota, and Julie Tourmetz, for the collection of biological material in the field. This work was funded by the Structure fédérative BioSécurité en milieu Tropical and by the tutorship institutions of the Unité Mixte de Recherche, Processus Infectieux en Milieu Insulaire Tropical. Comments from reviewers helped us to clarify our manuscript.

REFERENCES

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., & Rohani, P. (2006). Seasonality and the dynamics of infectious diseases. *Ecology Letters, 9*(4): 467–484. https://doi.org/10.1111/j.1461-0248.2005.00879.x

Feare, C. J. (1976). The breeding of the Sooty tern *Sterna fuscata* in the Seychelles and the effects of experimental removal of its eggs. *Journal of Zoology, 179*(3), 317–360. https://doi.org/10.1111/j.1469-7998.1976.tb02299.x

Feare, C. J. (1979). Ecology of Bird Island, Seychelles. *Atoll Research Bulletin, 226*, 1–38. https://doi.org/10.5479/si.00775630.226.1

Joffrin, L., Goodman, S. M., Wilkinson, D. A., Ramasindrazana, B., Lagadec, E., Gomard, Y., Le Minter, G., Dos Santos, A., Schoeman, M. C., Sookhareea, R., Tortosa, P., Julienne, S., Gudo, E. S., Mavingui, P., & Lebarbenchon, C. (2020). Bat coronavirus phylogeography in the Western Indian Ocean. *Scientific Reports, 10*, Article 6873. https://doi.org/10.1038/s41598-020-63799-7

Joffrin, L., Hoarau, A. O. G., Lagadec, E., Torrontegi, O., Köster, M., Le Minter, G., Dietrich, M., Mavingui, P., & Lebarbenchon, C. (2022). Seasonality of coronavirus shedding in tropical bats. *Royal Society Open Science, 9*(2), Article 211600. https://doi.org/10.1098/rsos.211600

Le Corre, M., & Jaquemet, S. (2005). Assessment of the seabird community of the Mozambique Channel and its potential use as an indicator of tuna abundance. *Estuarine, Coastal and Shelf Science, 63*(3), 421–428. https://doi.org/10.1016/j.ecss.2004.11.013

Lebarbenchon, C., Boucher, S., Feare, C., Dietrich, M., Larose, C., Humeau, L., Le Corre, M., & Jaeger, A. (2023). Migratory patterns of two major influenza virus host species on tropical islands. *Royal Society Open Science, 10*(10), Article 230600. https://doi.org/10.1098/rsos.230600

Lebarbenchon, C., Jaeger, A., Bastien, M., Le Corre, M., Dellagi, K., & Pascalis, H. (2013). Absence of coronaviruses, paramyxoviruses, and influenza A viruses in seabirds in the Southwestern Indian Ocean. *Journal of Wildlife Diseases, 49*(4), 1056–1059. https://doi.org/10.7589/2012-09-227

Lebarbenchon, C., Jaeger, A., Feare, C., Bastien, M., Dietrich, M., Larose, C., Lagadec, E., Rocamora, G., Shah, N., Pascalis, H., Boulinier, T., Le Corre, M., Stallknecht, D. E., & Dellagi, K. (2015). Influenza A virus on oceanic islands: Host and viral diversity in seabirds in the Western Indian Ocean. *PLOS Pathogens, 11*(5), Article e1004925. https://doi.org/10.1371/journal.ppat.1004925

Lebarbenchon, C., Ramasindrazana, B., Joffrin, L., Bos, S., Lagadec, E., Le Minter, G., Gomard, Y., Tortosa, P., Wilkinson, D. A., Goodman, S. M., & Mavingui, P. (2017). Astroviruses in bats, Madagascar. *Emerging Microbes and Infections, 6*(1), Article e58. https://doi.org/10.1038/emi.2017.47

Muradrasoli, S., Mohamed, N., Hornyák, Á., Fohlman, J., Olsen, B., Belák, S., & Blomberg, J. (2009). Broadly targeted multiprobe QPCR for detection of coronaviruses: Coronavirus is common among Mallard Ducks (*Anas platyrhynchos*). *Journal of Virological Methods, 159*(2), 277–287. https://doi.org/10.1016/j.jviromet.2009.04.022

Ninove, L., Nougairede, A., Gazin, C., Thirion, L., Delogu, I., Zandotti, C., Charrel, R. N., & De Lamballerie, X. (2011). RNA and DNA bacteriophages as molecular diagnosis controls in clinical virology: A comprehensive study of more than 45,000 routine PCR tests. *PLOS One, 6*(2), Article e16142. https://doi.org/10.1371/journal.pone.0016142

Wille, M., Avril, A., Tolf, C., Schager, A., Larsson, S., Borg, O., Olsen, B., & Waldenström, J. (2015). Temporal dynamics, diversity, and interplay in three components of the virodiversity of a Mallard population: Influenza A virus, avian paramyxovirus and avian coronavirus. *Infection, Genetics and Evolution, 29*(January), 129–137. https://doi.org/10.1016/j.meegid.2014.11.014

Wille, M., & Holmes, E. C. (2020). Wild birds as reservoirs for diverse and abundant gamma- and deltacoronaviruses. *FEMS Microbiology Reviews, 44*(5), 631–644. https://doi.org/10.1093/femsre/fuaa026

Wille, M., Lindqvist, K., Muradrasoli, S., Olsen, B., & Järhult, J. D. (2017). Urbanization and the dynamics of RNA viruses in Mallards (*Anas platyrhynchos*). *Infection, Genetics and Evolution, 51*(July), 89–97. https://doi.org/10.1016/j.meegid.2017.03.019